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(54) Title: MODIFICATION OF ANGIOGENESIS BY TARGETING PROTEIN TYROSINE PHOSPHATASES

(57) Abstract: The present invention relates to a process for identifying a compound which is a modifier of angiogenesis, said process comprising contacting a cell or cell extract with said compound, determining whether there is a change in the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and thereby determinig whether the compounds is a modifier of angiogenesis.

Modification of Angiogenesis by Targeting Protein Tyrosine Phosphatases

Technical Field

The present invention relates to methods for screening chemical compounds to identify a compound which modifies angiogenesis through its interaction with at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B. In addition it relates to the use of the compounds so identified, and compositions containing same in the modification of these protein tyrosine phosphatases and the subsequent modification of angiogenesis, in particular the inhibition of angiogenesis. Further, the invention relates to methods of treating angiogenic dependent disease via the use of compounds capable of changing the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B.

Background Art

The sprouting of new capillaries from existing vessels is termed angiogenesis, and occurs during embryogenesis and in the adult. In the normal adult mammal angiogenesis is confined to the female reproductive cycles, wound healing and several pathological situations. Angiogenesis is a key factor in diseases such as rheumatoid arthritis, psoriasis, diabetic retinopathy and cancer.

Angiogenesis is controlled by the balance between proliferative/anti-apoptotic factors such as vascular endothelial cell and fibroblast growth factors and anti-proliferative/pro-apoptotic factors such as thrombospondin, angiostatin and endostatin. In response to proliferative factors, the basement membrane surrounding an endothelial cell tube is locally degraded, which triggers the endothelial cells underlying this disrupted matrix to change shape and invade the surrounding tumor stroma. The invading endothelial cells proliferate and develop into a migrating column. In response to anti-proliferative factors, the cells of the column wall stop proliferating, change shape, and adhere to each other to form the lumen of the new capillary. Ultimately, the new capillaries fuse and form into loops, resulting in a circulatory system that facilitates exchange of nutrients and waste products in the region.

Signalling within and between endothelial cells during angiogenesis is mediated in large part by phosphorylation or dephosphorylation of protein tyrosine residues. The level of cellular tyrosine phosphorylation is balanced by the activity of tyrosine kinases and protein tyrosine phosphatases.

Accordingly, there is a need to identify those compounds that perturb tyrosine phosphorylation through the modification of the activity of protein tyrosine phosphatases, and as a result, have an modifying effect on angiogenesis.

The present invention resides in the discovery that the modification in activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B has a modifying effect upon angiogenesis, particularly in proliferating endothelial cells. This discovery was exemplified by the observation that a substantially cell-membrane impermeable arsenoxide or arsenoxide equivalent compound modifies the activity of these enzymes, and this discovery thereby provides a means for screening other compounds that have a role in the modification of angiogenesis through their action on the activity of these protein tyrosine phosphatases and for the use of compounds capable of modifying the activity of these enzymes in the treatment of angiogenic dependent disease.

Therefore, the present invention provides a method of screening a plurality of candidate compounds to identify a compound which modifies at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B. Further, the present invention provides a method for modifying at least one such protein tyrosine phosphatase in a vertebrate, and thereby modifying angiogenesis, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one of the compounds identified by the screening method or at least one substantially cell-membrane impermeable arsenoxide or arsenoxide equivalent compound.

Summary of the Invention

According to a first embodiment of the invention, there is provided a process for identifying a compound which is a modifier of angiogenesis, said process comprising contacting a cell or cell extract with a candidate compound, determining whether there is a change in the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and thereby determining whether the compound is a modifier of angiogenesis.

According to a second embodiment of the invention, there is provided a process for screening a plurality of compounds to identify a compound which is a modifier of angiogenesis, wherein said process comprises contacting a cell or a cell extract with said plurality of compounds, determining whether there is a change in the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and if so, separately determining the change in activity of said protein tyrosine phosphatase for each of the plurality of compounds, thereby determining the identity of the compound which is a modifier of angiogenesis.

Typically, the protein tyrosine phosphatase is PTP-PEST. More typically, the protein tyrosine phosphatase is PTP-1B.

Typically, the cell is a mammalian cell. More typically, the cell is selected from the group consisting of: endothelial cells, epithelial cells, immune cells, bone, bone marrow cells and tumour cells. Even more typically, the cell is selected from the group consisting of: proliferating endothelial cells.

Typically, the process in accordance with the first or second embodiments of the invention comprises contacting a cell extract.

Typically, the compounds screened in accordance with the first or second embodiments of the invention are compounds that are not previously known to modify angiogenesis.

According to a third embodiment of the invention, there is provided the process of the first or second embodiment of the present invention, wherein said modifier inhibits angiogenesis.

According to a fourth embodiment of the invention, there is provided the process of the first or second embodiment of the present invention, wherein said modifier stimulates angiogenesis.

According to a fifth embodiment of the invention, there is provided a method of inhibiting at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B in a vertebrate, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier detected in accordance with the process of the third embodiment of the present invention, or a therapeutically effective amount of a pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

According to a sixth embodiment of the invention, there is provided use of a therapeutically effective amount of at least one modifier detected in accordance with the process of the third embodiment of the present invention for the manufacture of a medicament for inhibiting at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B in a vertebrate.

According to a seventh embodiment of the invention, there is provided a method of stimulating at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B in a vertebrate, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier detected in accordance with the process of the fourth embodiment of the present invention, or a therapeutically effective amount of a pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

According to an eighth embodiment of the invention, there is provided use of a therapeutically effective amount of at least one modifier detected in accordance with the process of the fourth embodiment of the present invention for the manufacture of a medicament for stimulating

at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B in a vertebrate.

According to a ninth embodiment of the invention, there is provided a method of inhibiting angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier detected in accordance with the process of the third embodiment of the present invention, or a therapeutically effective amount of a pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

According to a tenth embodiment of the invention, there is provided use of a therapeutically effective amount of at least one modifier detected in accordance with the process of the third embodiment of the present invention for the manufacture of a medicament for inhibiting angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B.

According to an eleventh embodiment of the invention, there is provided a method of stimulating angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier detected in accordance with the process of the fourth embodiment of the present invention, or a therapeutically effective amount of a pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

According to a twelfth embodiment of the invention, there is provided use of a therapeutically effective amount of at least one modifier detected in accordance with the process of the fourth embodiment of the present invention for the manufacture of a medicament for stimulating angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B.

According to a thirteenth embodiment of the invention, there is provided a method of treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier detected in accordance with the process of the third embodiment of the present invention, or a therapeutically effective amount of a

pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

According to a fourteenth embodiment of the invention, there is provided use of a therapeutically effective amount of at least one modifier detected in accordance with the process of the third embodiment of the present invention for the manufacture of a medicament for treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B.

According to a fifteenth embodiment of the invention, there is provided a method of modifying angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and wherein said method comprises administering to the vertebrate a therapeutically effective amount of an compound capable of changing the activity of at least one of said protein tyrosine phosphatases.

According to a sixteenth embodiment of the invention, there is provided use of a therapeutically effective amount of an compound capable of changing the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B for the manufacture of a medicament for modifying angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B.

Typically, angiogenesis is inhibited or stimulated. More typically, the activity of said protein tyrosine phosphatase is inhibited or stimulated.

According to a seventeenth embodiment of the invention, there is provided a method of treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and wherein said method comprises administering a therapeutically effective amount of at least one compound capable of inhibiting the activity of at least one of said protein tyrosine phosphatases.

According to a eighteenth embodiment of the invention, there is provided use of a therapeutically effective amount of at least one compound capable of inhibiting the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B for the manufacture of a medicament for treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B.

According to a nineteenth embodiment of the invention, there is provided a method of treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and wherein said method comprises administering a therapeutically effective amount of at least one compound capable of stimulating the activity of at least one of said protein tyrosine phosphatases.

According to a twentieth embodiment of the invention, there is provided use of a therapeutically effective amount of at least one compound capable of stimulating the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B for the manufacture of a medicament for treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B.

According to a twenty-first embodiment of the invention, there is provided a method of inhibiting angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one substantially cell-membrane impermeable arsenoxide or arsenoxide equivalent compound.

According to a twenty-second embodiment of the invention, there is provided use of a therapeutically effective amount of at least one substantially cell-membrane impermeable arsenoxide or arsenoxide equivalent compound for the manufacture of a medicament for inhibiting angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B.

According to a twenty-third embodiment of the invention, there is provided a method of treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one substantially cell-membrane impermeable arsenoxide or arsenoxide equivalent compound.

According to a twenty-fourth embodiment of the invention, there is provided use of at least one substantially cell-membrane impermeable arsenoxide or arsenoxide equivalent compound for the manufacture of a medicament for treating an angiogenesis dependent disease, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase in a vertebrate in a vertebrate.

Typically, in relation to any one of the fifth through to twenty-fourth embodiments of the invention, the vertebrate is one in need of said modification, inhibition or treatment.

In relation to any one of the nineteenth to twenty-fourth embodiments of the invention, the substantially cell-membrane impermeable compound typically targets rapidly proliferating endothelial cells and is of the formula (I):

$$A\text{-}(L\text{-}Y)_p \qquad (I)$$

wherein

A comprises at least one substantially cell-membrane impermeable pendant group;

L comprises any suitable linker and/or spacer group;

Y comprises at least one arsenoxide or arsenoxide equivalent;

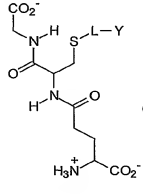
p is an integer from 1 to 10.

Typically, the sum total of carbon atoms in A and L together, is greater than 6.

With reference to the substantially cell-membrane impermeable compound of formula (I) suitable for use in the present invention, typically, A is selected from the group consisting of natural, unnatural and synthetic amino acids, hydrophilic amines, peptides, polypeptides, oligosaccharides, and thiol containing proteins, or a combination thereof. More typically, A is selected from the group consisting of glutathione, glucosamine, cysteinylglycine, cysteic acid, aspartic acid, glutamic acid, lysine, and arginine, and wherein the sulfur atom of each sulfur containing compound may be optionally oxidised to form a sulfoxide or sulfone.

Amino acid side chains are known to those of skill in the art and are listed, for example, in standard reference texts such as King and Stansfield, "A Dictionary of Genetics", 4th Edition, Oxford University Press, 1990, the contents of which are incorporated herein by reference.

Even more typically, A is glutathione and in one form the substantially cell-membrane impermeable compound suitable for use in the present invention is represented by Formula (II):



(II)

wherein L comprises any suitable linker and/or spacer group, and Y comprises an arsenoxide or an arsenoxide equivalent.

Typically, p is an integer from 1 to 5. Yet still more typically, p is 1.

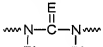
Typically, L corresponds to $(XBX')_nB'$. Typically, n is an integer from 0 to 20, more typically 0 to 15, even more typically 0 to 10, still more typically 0 to 5.

Still in accordance with the substantially cell-membrane impermeable arsenoxide compounds suitable for use in the present invention, the following relates to $(XBX')_nB'$.

Typically, X is selected from the group consisting of –NR, -S(O)-, -S(O)O-, -S(O)$_2$-, -S(O)$_2$O-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, -P(O)(R_1)-, and -P(O)(R_1)O-, or is absent;

B is selected from the group consisting of C_1-C_{10} alkylene, C_2-C_{10} alkenylene, C_2-C_{10} alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_3-C_{10} heterocycloalkylene, C_5-C_{10} heterocycloalkenylene, C_6-C_{12} arylene, heteroarylene and C_2-C_{10} acyl;

X' is selected from the group consisting of -NR-, –O-, -S-, -Se-, -S-S-, S(O)-, -OS(O)-, OS(O)O-, -OS(O)$_2$, -OS(O)$_2$O-, -S(O)O-, -S(O)$_2$-, -S(O)$_2$O-, -OP(O)(R_1)-, -OP(O)(R_1)O-, -OP(O)(R_1)OP(O)(R_1)O-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, -P(O)(R_1)-, -P(O)(R_1)O-, and



or is absent; wherein E is O, S, Se, NR or N(R)$_2^+$;

n is 0, 1 or 2; and

B' is selected from the group consisting of C_1-C_{10} alkylene, C_2-C_{10} alkenylene, C_2-C_{10} alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_3-C_{10} heterocycloalkylene, C_5-C_{10} heterocycloalkenylene, C_6-C_{12} arylene, and heteroarylene or is absent; and wherein

each R is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, OR_2 and C_2-C_{10} acyl;

R' is the same as R or two R' may be taken together with the nitrogen atoms to which they are attached to form a 5 or 6-membered saturated or unsaturated heterocyclic ring;

each R_1 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, halo, OR_2 and N(R)$_2$;

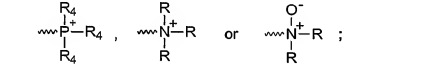
each R_2 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl and -C(O)R_5;

each R_5 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy,

C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, OH, SH and $N(R)_2$;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent) may be in a para-, meta- or ortho- relationship; and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, heterocycloalkylene, heterocycloalkenylene, arylene, heteroarylene and acyl may be independently substituted with hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, cyano, cyanate, isocyanate, OR_{2a}, SR_5, nitro, arsenoxide, -S(O)R_3, -OS(O)R_3, -S(O)$_2R_3$, -OS(O)$_2R_3$, -P(O)R_4R_4, -OP(O)R_4R_4, -N(R")$_2$, -NRC(O)$(CH_2)_m$Q, -C(O)R_5;



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, -S(O)R_3, -S(O)$_2R_3$, -P(O)$(R_4)_2$, $N(R)_2$ and -C(O)R_5;

each R_3 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio and $N(R)_2$;

each R_4 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, halo and $N(R)_2$;

R_6 is selected from the group consisting of C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, $-S(O)R_3$, $-S(O)_2R_3$ and $-C(O)R_5$;

R" is the same as R or two R" taken together with the N atom to which they are attached may form a saturated, unsaturated or aromatic heterocyclic ring system;

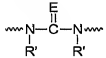
Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

More typically, X is selected from the group consisting of -C(O)-, -C(S)-, -C(O)O-, C(S)O-, and -C(S)S-, or is absent;

B is selected from the group consisting of C_1-C_5 alkylene, C_2-C_5 alkenylene, C_2-C_5 alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_6-C_{12} arylene and C_2-C_5 acyl;

X' is selected from the group consisting of –O-, -S-, -NR-, -S-S-, -S(O)-, -S(O)$_2$-, -P(O)(R_1)-, -OP(O)(R_1)-, OP(O)(R_1)O-, -OP(O)(R_1)OP(O)(R_1)O-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, -Se-,



, or is absent; wherein E is O, S or N(R)$_2$*;

n is 0, 1 or 2; and

B' is C_1-C_5 selected from the group consisting of alkylene, C_2-C_5 alkenylene, C_2-C_5 alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, and C_6-C_{12} arylene, or is absent; and wherein

each R is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, OR_2 and C_2-C_{10} acyl;

R' is the same as R;

each R_1 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, OR_2 and $N(R)_2$;

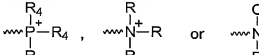
each R_2 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, and $-C(O)R_5$;

each R_5 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5

alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, OH, SH, and $N(R)_2$;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent), may be in a para-, meta- or ortho- relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, cyano, halo, cyanate, isocyanate, OR_{2a}, SR_5, nitro, arsenoxide, $-S(O)R_3$, $-OS(O)R_3$, $-S(O)_2R_3$, $-OS(O)_2R_3$, $-P(O)R_4R_4$, $-OP(O)R_4R_4$, $-N(R'')_2$, $NRC(O)(CH_2)_mQ$, $-C(O)R_5$,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)(R_4)_2$, $N(R)_2$ and $-C(O)R_5$;

each R_3 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio and $N(R)_2$;

each R_4 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_5 cycloalkylthio, C_5-C_5 cycloalkenylthio, C_6-C_{12} arylthio, halo and $N(R)_2$;

R_6 is independently selected from the group consisting of C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, $-S(O)R_3$, $-S(O)_2R_3$ and $-C(O)R_5$,

R'' is the same as R;

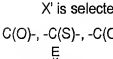
Q is selected from the group consisting of halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

Even more typically, X is absent;

B is selected from the group consisting of C_1-C_5 alkylene, C_6-C_{12} arylene and C_2-C_5 acyl;

X' is selected from the group consisting of –O-, -S-, -NR-, -S-S-, -S(O)-, -$S(O)_2$-, -P(O)(R_1)-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -Se-, and



, or absent; wherein E is O, S or $N(R)_2^+$;

n is 0, 1 or 2; and

B' is C_1-C_5 alkylene, C_6-C_{12} arylene or is absent; and wherein

each R is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, OR_2 and C_2-C_5 acyl;

R' is the same as R;

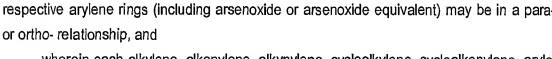
each R_1 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, halo, OR_2 and $N(R)_2$;

each R_2 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl and -C(O)R_5;

each R_5 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, OH, SH and $N(R)_2$;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent) may be in a para-, meta- or ortho- relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, cyano, cyanate, isocyanate, OR_2, SR_5, nitro, arsenoxide, -S(O)R_3, -OS(O)R_3, -$S(O)_2R_3$, -$OS(O)_2R_3$, -P(O)R_4R_4, -OP(O)R_4R_4, -N(R")$_2$, -NRC(O)$(CH_2)_m$Q, -C(O)R_5,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, -S(O)R_3, -S(O)$_2R_3$, -P(O)$(R_4)_2$ and –C(O)R_5;

each R_3 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_{10} cycloalkyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_{10} cycloalkylthio, C_6-C_{12} arylthio and $N(R)_2$;

each R_4 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_{10} cycloalkyloxy, C_6-C_{12} aryloxy, halo and $N(R)_2$;

R_6 is selected from the group consisting of C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_3-C_{10} cycloalkylthio, C_6-C_{12} arylthio, -S(O)R_3, -S(O)$_2R_3$ and –C(O)R_5,

R" is the same as R;

Q is selected from halogen and –OS(O)$_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

Still more typically, X is absent;

B is selected from the group consisting of C_1-C_5 alkylene, C_6-C_{12} arylene and C_2-C_5 acyl;

X' is selected from the group consisting of –O-, -S-, -NR-, -C(O)-, and -C(O)O-, or is absent;

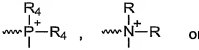
n is 1; and

B' is C_1-C_5 alkylene, C_6-C_{12} arylene or is absent; and

R is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl and C_2-C_5 acyl;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent), may be in a para-, meta- or ortho- relationship, and

wherein each alkylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, cyano, cyanate, isocyanate, OR_{2a}, SR_6, nitro, arsenoxide, -S(O)R_3, -S(O)$_2R_3$, -P(O)R_4R_4, -N(R")$_2$, -NRC(O)$(CH_2)_m$Q, -C(O)R_5,



wherein each R is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl and C_2-C_5 acyl;

R_{2a} is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, -S(O)R_3, -S(O)$_2R_3$, -P(O)$(R_4)_2$ and -C(O)R_5;

each R_3 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, and C_6-C_{12} arylthio;

each R_4 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_6-C_{12} arylthio, halo and $N(R)_2$;

each R_5 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_6-C_{12} arylthio, OH, SH and $N(R)_2$;

R_6 is selected from the group consisting of C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_6-C_{12} arylthio, $-S(O)R_3$, $-S(O)_2R_3$ and $-C(O)R_5$,

R" is the same as R above;

Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

Yet still more typically, X is absent;

B is C_2-C_5 acyl;

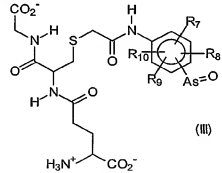
X' is NR;

n is 1;

B' is phenylene; and

R is H;

wherein the substituents directly attached to the phenylene ring may be in a para-, meta- or ortho- relationship, as exemplified by Formula (III):



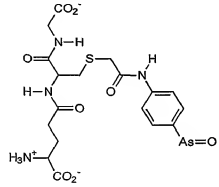
(III)

wherein R_7 to R_{10} are independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, $-OS(O)_2R_3$ and $-NHC(O)CH_2Q$ wherein Q is halogen, $-OS(O)_2CH_3$, $-OS(O)_2C_6H_5$ and $-OS(O)_2$-*p* tolyl; and wherein, when any one of R_7 to R_{10} is C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, $-OS(O)_2R_3$ it is capable of forming a fused ring with the phenylene; and further wherein, at least one of R_7 to R_{10} is C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, or $-OS(O)_2R_3$, in combination with at least any one other of R_7 to R_{10}, is capable of forming a fused ring with the phenylene.

More typically, R_7 to R_{10} are independently selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, cyano, carboxy, C_1-C_5 alkoxy, methyl, ethyl, isopropyl, tert-butyl, phenyl and $-NHC(O)CH_2Q$ wherein Q is halogen, $-OS(O)_2CH_3$, $-OS(O)_2C_6H_5$ and $-OS(O)_2$-*p* tolyl.

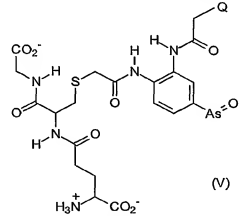
Further, when B' is arylene, the substituents attached to the arylene ring are typically in an ortho-,meta- or para- relationship to the –As=O. More typically the substituents are in a meta- or para- relationship to the –As=O group.

More preferably a substantially cell-membrane impermeable arsenoxide compound suitable for use in the present invention is 4-(*N*-(*S*-glutathionylacetyl)amino)-phenylarsenoxide, which can be abbreviated to GSAO, according to Formula IV:



(IV)

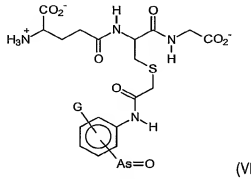
Other substantially cell membrane impermeable arsenoxide compounds suitable for use in the present invention include compounds according to Formula (V):



(V)

wherein Q is any halogen. For example, the invention provides the compounds 3-(*N*-(fluoroacetyl)amino)-4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide, which may be abbreviated to GSFAO, 3-(*N*-(chloroacetyl)amino)-4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide, which may be abbreviated to GSCAO, 3-(*N*-(bromoacetyl)amino)-4-(*N*-(*S*-glutathionylacetyl)amino)-phenylarsenoxide, which may be abbreviated to GSBAO, and 3-(*N*-(iodoacetyl)amino)-4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide, which may be abbreviated to GSIAO.

Another preferred form of the arsenoxide compounds suitable for use in the invention is a compound according to Formula (VI):



(VI)

wherein G is selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, cyano, carboxy, C_1-C_5 alkoxy, C_1-C_5 alkyl and C_6-C_{12} aryl and -NHC(O)CH_2Q, wherein Q is halogen, -OS(O)$_2CH_3$, -OS(O)$_2C_6H_5$ or –OS(O)$_2$-*p* tolyl.

Typically, G is selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, methyl, ethyl, iso-propyl, tert-butyl, phenyl, and -NHC(O)CH_2Q, wherein Q is the group consisting of halogen, -OS(O)$_2CH_3$, -OS(O)$_2C_6H_5$ and –OS(O)$_2$-*p* tolyl.

More typically, in a compound of Formula VI, G is hydroxy, fluorine, amino, or nitro.

Typically, group G is in an ortho-, meta- or para- relationship to the arsenoxide group, more typically an ortho- or para- relationship.

Typically the activity of the arsenic atom may be modified by the group G, when G and the arsenic atom are in an ortho or para relationship to one another. For example, when G is an electron donating group such as OH (ionised to O$^-$ at physiological pH), the arsenic atom should be deactivated towards dithiols and so become more selective, only reacting with very reactive dithiols. Alternatively, when G is an electron withdrawing group, such as NO_2, electron density would be drawn away from the arsenic atom, making it more reactive to all dithiols. Selective inhibition of some redox proteins and not others may be achieved by manipulation of G.

Typically, in the arsenoxide compounds suitable for use in the present invention, the arsenoxide group (-As=O) can be replaced by an arsenoxide equivalent.

An arsenoxide equivalent is any dithiol reactive species that shows essentially the same affinity towards dithiols as –As=O. Typically, arsenoxide equivalent includes dithiol reactive entities, such as As, Ge, Sn and Sb species. More typically an arsenoxide equivalent can be represented by -D(Z_1)(Z_2). Arsenoxide equivalents are expected to exhibit identical or substantially identical activity to that of the corresponding arsenoxide.

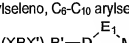
Typically, for arsenoxide equivalents of the form -D(Z_1)(Z_2), D will be, for example, As RSn, Sb, or RGe, and Z_1 and Z_2 will be labile groups (i.e. groups easily displaced under physiological

conditions). Z_1 and Z_2, may be identical or different, and may either be connected or independent from each other (bound only to the arsenic atom).

Suitable arsenoxide equivalents include the following:

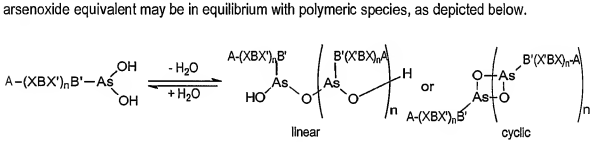
$$-D(Z_1)(Z_2),$$

wherein Z_1 and Z_2 are selected from the group consisting of OH, C_1-C_{10} alkoxy, C_6-C_{10} aryloxy, C_1-C_{10} alkylthio, C_6-C_{10} arylthio, C_1-C_{10} alkylseleno, C_6-C_{10} arylseleno, F, Cl, Br and I;

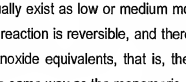


wherein E_1 = E_2 = O, E_1 =O and E_2 = S or E_1 = E_2 = S; M is R''' and R'''' are independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_6-C_{12} aryl, halogen, C_1-C_{10} alkoxy, C_6-C_{10} aryloxy, hydroxy and carboxy; and n = 1 to 10.

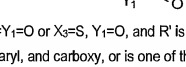
For arsenoxide equivalents of the form $D(Z_1)(Z_2)$, when D is As and Z_1 and Z_2 are OH, the arsenoxide equivalent may be in equilibrium with polymeric species, as depicted below.

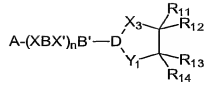


In respect of the equilibrium depicted above, arsenic is one of many elements whose hydroxy species exist in equilibrium with the corresponding polymeric anhydrides. Therefore, arsenoxide compounds may actually exist as low or medium molecular weight polymers (eg n = 3 to 6). However, the dehydration reaction is reversible, and therefore soluble polymeric anhydrides are expected to behave as arsenoxide equivalents, that is, they are expected to bind to closely spaced dithiols in substantially the same way as the monomeric $-As(OH)_2$ species.

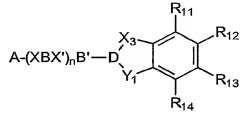


wherein X_3=NH, Y_1=O; X_3=Y_1=O or X_3=S, Y_1=O, and R' is selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_6-C_{12} aryl, and carboxy, or is one of the twenty amino acid side chains;





wherein X_3=Y_1=O; X_3=NH, Y_1=O; X_3=S, Y_1=O; X_3=Y_1=NH; or X_3=S, Y_1=NH; or X_3=S, Y_1=NH and R_{11} to R_{14} are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_6-C_{12} aryl, and CO_2H;



wherein X_3=Y_1=O, or X_3=NH, Y_1=O; and R_{11} to R_{14} are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_6-C_{12} aryl, halogen, C_1-C_{10} alkoxy, and CO_2H.

Typically, (XBX')B' is as defined above.

Definitions

In the context of this specification, the term "comprising" means "including principally, but not necessarily solely". Furthermore, variations of the word "comprising", such as "comprise" and "comprises", have correspondingly varied meanings.

In the context of this specification, the term "arsenoxide" refers to the group –As=O.

In the context of this specification, the groups written –As=O and $-As(OH)_2$ are to be considered synonymous.

In the context of this specification, the term "arsenoxide equivalent" refers to any dithiol reactive species that shows essentially the same affinity towards dithiols as –As=O or $As(OH)_2$, and the term includes, for example, groups comprising a transition element, and any trivalent arsenical that is either hydrolysed to –As=O or $-As(OH)_2$ when dissolved in an aqueous medium (such as cell culture buffers and the fluids contained in the organism being treated).

The term "arsenical" as used herein, includes any compound that contains arsenic.

The term "acyl" as used herein, includes monovalent and divalent alkyl, alkenyl, alkynyl, cycloalkyl and cycloalkenyl moieties possessing a terminal carbonyl substituent wherein attachment may occur at the hydrocarbon moiety, the carbonyl moiety or both.

The term "alkyl" as used herein, includes within its meaning monovalent, saturated, straight and branched chain hydrocarbon radicals.

The term "alkenyl" as used herein, includes within its meaning, monovalent, straight and branched chain hydrocarbon radicals having at least one double bond.

The term "alkynyl" as used herein, includes within its meaning, monovalent, straight and branched chain hydrocarbon radicals having at least one triple bond.

The term "alkylene" as used herein, includes within its meaning divalent, saturated, straight chain hydrocarbon radicals.

The term "alkenylene" as used herein, includes within its meaning, divalent, straight chain hydrocarbon radicals having at least one double bond.

The term "alkynylene" as used herein, includes within its meaning, divalent, straight chain hydrocarbon radicals having at least one triple bond.

The term "aryl" as used herein, includes within its meaning monovalent, single, polynuclear, conjugated and fused aromatic hydrocarbon radicals.

The term "arylene" as used herein, includes within its meaning divalent, single, polynuclear, conjugated and fused aromatic hydrocarbon radicals.

The term "closely spaced dithiol" as used herein, includes within its meaning thiols that are chemically vicinal, as well as thiols brought into spacial apposition by virtue of molecular conformation.

The term "cycloalkyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals.

The term "cycloalkylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals.

The term "cycloalkenyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals having at least one double bond.

The term "cycloalkenylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals having at least one double bond.

The term "halo" as used herein, includes fluoro, chloro, bromo and iodo.

The term "heteroaryl" as used herein, includes within its meaning monovalent, single, polynuclear, conjugated and fused aromatic radicals having 1 to 12 atoms wherein 1 to 6 atoms are heteroatoms selected from O, N and S.

The term "heteroarylene" as used herein, includes within its meaning divalent, single, polynuclear, conjugated and fused aromatic radicals having 1 to 12 atoms wherein 1 to 6 atoms are heteroatoms selected from O, N and S.

The term "heterocycloalkyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused radicals wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "heterocycloalkylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic radicals wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "heterocycloalkenyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic radicals having at least 1 double bond and wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "heterocycloalkenylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic radicals having at least one double bond and wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "phenylarsonic acid" as used herein, is to be considered synonymous with "benzene sulfonic acid".

The term "therapeutically effective amount" as used herein, includes within its meaning a non-toxic but sufficient amount a compound or composition for use in the invention to provide the desired therapeutic effect. The exact amount required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of the subject, the severity of the condition being treated, the particular agent being administered and the mode of administration and so forth. Thus, it is not possible to specify an exact "effective amount". However, for any given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

The term "transition element" as used herein, includes within its meaning the groups of elements comprising the transition metals, the lanthanides and the actinides.

Abbreviations

pAA, *p*-arsanilic acid, 4-aminobenzenearsonic acid; AspAO, *N*-(3-(4-arsenosophenylcarbamoylmethylthio)propanoyl)-L-aspartic acid; BAE, bovine aortic endothelial; BCE, bovine capillary endothelial; BCS, bovine calf serum; BSA, bovine serum albumin; BAE, bovine aortic endothelial; BCE, bovine capillary endothelial; BVSM, bovine vascular smooth muscle; BRAA, 4-(*N*-(bromoacetyl)amino)phenylarsonic acid; BRAO, 4-(*N*-(bromoacetyl)amino)-phenylarsenoxide; BVS, bovine vascular smooth muscle; CAM, chick chorioallantoic membrane; Cys*AO, *N*-(3-(4-arsenosophenylcarbamoylmethylthio)propanoyl)-L-cysteic acid; DMEM, Dulbecco's Modified Eagle's Medium; DMP, 2,3-dimercaptopropanol; DMSO, dimethylsulfoxide; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic

acid; FAK, focal adhesion kinase; FAKpTyr, tyrosine phosphorylated FAK; GSCA, (4-(N-(S-glutathionylacetyl)amino)benzoic acid); FCS, fetal calf serum; FGF, fibroblast growth factor; GSAO-F, 4-(*N*-(*S*-(*N*-(3-(fluorescein-5-carbamoylmethylthio)propanoyl)glutathionyl)acetyl)amino)phenylarsenoxide; FXAO, a mixture of 4-(*N*-(6-(fluorescein-5-carboxamido)hexanoyl)amino)phenylarsenoxide and 4-(*N*-(6-(fluorescein-6-carboxamido)hexanoyl)amino)phenylarsenoxide; GlcAO, *N*-(3-(4-arsenosophenylcarbamoylmethylthio)propanoyl)-D-glucosamine; GluAO, *N*-(3-(4-arsenosophenylcarbamoylmethylthio)propanoyl)-L-glutamic acid; GSAA, 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsonic acid; GSAO, 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide; GSAO-B, 4-(*N*-(*S*-(*N*-(6-(*N*-(6-(*N*-(biotinoyl)amino)hexanoyl)amino)hexanoyl)glutathionyl)acetyl)amino)phenylarsenoxide; GSH, reduced glutathione; HDMVEC, human dermal microvascular endothelial cell; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*'-(2-ethanesulfonic acid); HRP, horse-radish peroxidase; HUVEC, human umbilical vein endothelial cell; Ig, immunoglobulin; MPB, 3-(*N*-maleimidylpropionyl)biocytin; PAO, phenylarsenoxide; pAPAO, 4-aminophenylarsenoxide; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PAO, phenylarsenoxide; PTPase, phosphotyrosine phosphatase; PDI, protein disulfide isomerase; PVDF, polyvinyldiethylene fluoride; SCID, severe combined immunodeficient; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; SSB, sulfosuccinimidobiotin; TCR, T cell receptor; TNB, 5-thio-2-nitrobenzoate dianion; VEGF, vascular endothelial cell growth factor.

Brief Description of the Drawings

Figure 1. GSAO Slowly Entered Endothelial Cells Which Resulted in Cell Detachment

A Structures of PAO and GSAO. **B** Coupling PAO to reduced glutathione increased cytotoxic IC_{50} for endothelial cells by ~2,000-fold. 20,000 BAE cells per well were seeded into 96-well culture plates and allowed to attach for 24 h. The media was then replaced with DMEM and 10% FCS containing increasing concentrations of PAO, GSAO or GSAA. Cells were cultured for 24 h and the number of adherent cells was measured. The data points are the mean ± SE of triplicate wells. **C** GSAO did not reduce the viability of endothelial cells by interacting with cell-surface dithiols. 20,000 HMEC-1 per well were seeded into 96-well culture plates and allowed to attach for 24 h. The media was then replaced with MCDB and 10% FCS containing either 100 or 10 μM MPB or DTNB and incubated for 5 min. 0, 100 or 250 μM GSAO was then added and the cells cultured for a further 24 h. The number of adherent cells was measured. The data points are the mean ± SE of quadruplicate wells. **D** Time dependence of the affect of GSAO on endothelial cells. 50,000

BCE cells per well were seeded into gelatinized 24-well culture plates and allowed to attach for 24 h. The media was then replaced with DMEM containing 5% BCS and 1 ng.mL^{-1} FGF-2 and either 10 μM GSAO or GSAA. Cells were cultured for discrete times up to 48 h and then dispersed and counted. The dotted line represents no change in cell number over the 48 h. The data points are the mean ± range of duplicate wells. **E** Kinetics of uptake of GSAO. 500,000 HMEC-1 per well were seeded into 6-well culture plates and allowed to attach for 24 h. The media was then replaced with MCDB-131 and 10% FCS containing 3 μM ^{3}H-GSAO and the cells incubated for 24, 48 or 72 h. The cells were counted, lysed by repeated freeze/thawing and the amount of cytosolic ^{3}H-GSAO was determined. The data points are the mean ± SE quadruplicate wells.

Figure 2. GSAO Inhibited Dephosphorylation of FAK and Paxillin in Endothelial Cells and Bound to PTP-PEST and PTP-1B in Endothelial Cell Lysate

A GSAO caused hyperphosphorylation of tyrosine residues in focal adhesion proteins in endothelial cells. 80,000 HMEC-1 per well were seeded into 2-well Labtek glass chamber slides and allowed to attach for 24 h. The media was then replaced with MCDB-131 containing 10% FCS without or with 100 μM GSAO and the cells incubated for 10 h. The cells were washed and immunostained for phosphotyrosine. **B** HMEC-1 were untreated (lane 1) or incubated with 100 μM GSAO for 8 h (lane 2), 10 μM GSAO for 24 (lane 3), 48 (lane 4) or 72 (lane 5) h or 1 μM PAO for 4 h (lane 6). Cell lysate was prepared in the presence of 1 mM vanadate to block dephosphorylation and 25 μg resolved on 8-16% SDS-PAGE, transferred to PVDF membrane and blotted for phosphotyrosine. The extent of tyrosine phosphorylation of two proteins with molecular masses of ~120 and ~70 kDa was changed by incubation with GSAO (indicated by arrows). **C** HMEC-1 were untreated or incubated with 100 μM GSAO for 8 h. FAK or paxillin was immunoprecipitated from the cell lysates and blotted for phosphotyrosine. The membranes were stripped and reprobed for FAK or paxillin protein. The relative increase in tyrosine phosphorylation of FAK or paxillin is shown by the bar graph. **D** Phosphorylated FAK (FAKpTyr) was prepared in HMEC-1 by incubation with 10 μM PAO for 30 min and collected by immunoprecipitation. 50 μg of lysate from untreated HMEC-1 was incubated without or with 2 mM vanadate, 1 μM PAO or 0.1, 2 or 40 μM GSAO for 10 min at 37°C. FAKpTyr was then added to the lysates and incubated for 90 min at 37°C. Dephosphorylation of FAKpTyr was measured by blotting for phosphotyrosine. Densitometric analysis of the blots is shown by the bar graph. **E** HMEC-1 were incubated in the absence or presence of 100 μM GSAO for 8 h. Lysate was prepared from the untreated or treated cells and 50 μg incubated with FAKpTyr for 90 min at 37°C. Dephosphorylation of FAKpTyr was measured by blotting for phosphotyrosine. Densitometric analysis of the blots is shown by the bar graph. **F** Lysate of HMEC-1 was incubated with 2 μM GSAO-biotin in the absence or presence of

1 mM DMP for 1 h at 4ºC. PTP-PEST, SHP2, PTEN or PTP-1B was immunoprecipitated from the lysate and blotted with streptavidin-peroxidase to detect bound GSAO-biotin.

Figure 3. GSAO was a Selective Inhibitor of Endothelial Cell Proliferation and Viability

A-C 15,000 BCE (parts A and B) or BxPC-3 (part C) cells per well were seeded into 24-well culture plates and allowed to attach for 24 h. Gelatinized wells were used for BCE cells. The media was then replaced with DMEM containing 0 to 1 mM GSAO or GSAA and a mitogen. For BCE cells the mitogen was 5% BCS with either 1 ng.mL^{-1} FGF-2 (part A) or 10 ng.mL^{-1} VEGF (part B). For BxPC-3 cells the mitogen was 5% FCS. Cells were cultured for 72 h and adherent cells were dispersed and counted. The dotted lines indicate the cell number in control wells containing DMEM and either 5% BCS (parts A and B) or 2% FCS (part C), which represented no to limited proliferation. The data points are the mean ± range of duplicate wells. **D** 20,000 BAE cells per well were seeded into 96-well culture plates and allowed to attach for 24 h. The cells were made growth quiescent by incubating with DMEM containing 0.25% FCS for 48 h. The medium was then replaced with DMEM containing either 0.25% or 10% FCS and either 50 μM GSAO or 0.5 μM PAO. Cells were cultured for a further 48 h and the number of adherent cells was measured. The data points are the mean ± SE of quadruplicate wells.

Figure 4. GSAO Inhibited CAM Angiogenesis

Methylcellulose discs containing either GSAA or GSAO were applied to the CAM of individual embryos and incubated for 48 h. The number out of 5 zones positive for angiogenesis inhibition is shown in part A. GSAA did not inhibit CAM angiogenesis up to 50 μg per pellet. Photographs of CAM's after incubation with discs containing 10 μg of either GSAA (top) or GSAO (bottom) is shown in part B. The dotted circle indicates the placement of the disc.

Figure 5. GSAO Inhibited Tumor Angiogenesis and Tumor Growth in Mice

A-D SCID mice bearing ~0.1 g BxPC-3 (parts A and B) or HT1080 (part C) tumors or C57Bl6/J mice bearing ~0.1 g LLC tumors (part D) were randomized into two groups (n = 4 or 5) and treated with either GSAA or GSAO in 0.2 mL of PBS containing 100 mM glycine at the doses indicated. The compounds were administered S.C. at a site distant from the tumor. Tumor volume was measured as indicated. The GSAO/GSAA tumor volume ratios at the conclusion of treatment were 0.34 (part A), 0.09 (part B), 0.29 (part C) and 0.29 (part D). The data points are the mean ± SE of the tumor volumes. **E-F** Histological sections of the BxPC-3 tumors from the experiment shown in part B at day 31 of treatment with GSAA or GSAO were analyzed for angiogenesis (CD31), proliferation (PCNA) and apoptosis (TUNEL). An example of the staining for CD31 is shown in part

E and the analysis for blood vessel density and tumor cell proliferation and apoptosis is shown in part F.

Figure 6. GSAO in the Drinking Water Inhibited Human and Murine Primary Tumor Growth SCID mice bearing ~0.1 g AsPC-1 tumors (part A) or C57Bl6/J mice bearing ~0.1 g LLC tumors (part B) were randomized into two groups (n = 4) and treated with either GSAO or GSCA/GSAA (0.05 mg.mL^{-1}) in their water. Mice drink ~5 mL of water per day and therefore consumed ~10 mg GSAO or GSCA/GSAA per kg per day. The water contained 20 mM glycine to minimize oxidation of GSAO. Tumor volume was measured as indicated. The data points are the mean ± SE of the tumor volumes.

Figure 7. Schematic representation of the synthesis of GSAO-B

Figure 8. Schematic representation of the synthesis of GSAO

Figure 9. Schematic representation of the synthesis of GSAA

Best Mode of Performing the Invention

The present invention relates to a process of determining whether a compound is a modifier of angiogenesis, said process comprising contacting a cell or cell extract with said compound, determining whether there is a change in the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and thereby determining whether the compound is a modifier of angiogenesis.

In addition, the invention relates to a process for screening a plurality of compounds to identify a compound which is a modifier of angiogenesis, wherein said process comprises contacting a cell or a cell extract with said plurality of compounds, determining whether there is a change in the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and if so, separately determining the change in activity of said protein tyrosine phosphatase for each of the plurality of compounds, thereby determining the identity of the compound which is a modifier of angiogenesis.

Typically, the cell is a mammalian cell. More typically, the cell is selected from the group consisting of: endothelial cells, epithelial cells, immune cells, bone and bone marrow cells and tumour cells. Even more typically, the cell is selected from the group consisting of: proliferating endothelial cells.

Typically, the compounds screened in the processes of the invention are those that are not previously known to inhibit a protein tyrosine phosphatase, and more typically, those that are not

previously known to inhibit a protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B.

Further, the invention relates to a method of inhibiting or stimulating angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier of angiogenesis as detected above, or a therapeutically effective amount of a pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

The invention is also concerned with a method of treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier of angiogenesis as detected above, or a therapeutically effective amount of a pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

The invention also relates to a method of modifying angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and wherein said method comprises administering to the vertebrate a therapeutically effective amount of an compound capable of changing the activity of at least one of said protein tyrosine phosphatases.

The modification of angiogenesis can take the form of inhibition or stimulation. Further, the activity of said protein tyrosine phosphatase is inhibited or stimulated.

In addition the invention relates to a method of treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and wherein said method comprises administering a therapeutically effective amount of at least one compound capable of inhibiting or stimulating the activity of at least one of said protein tyrosine phosphatases.

In one aspect of the invention, the compound referred to above comprises at least one substantially cell-membrane impermeable arsenoxide or arsenoxide equivalent compound.

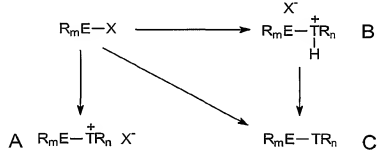
Synthesis of GSAO

As set out in International (PCT) Patent Application No. PCT/AU00/011434 (WO 01/21628), the disclosure of which is incorporated herein by reference, substantially cell-membrane

impermeable arsenoxide or arsenoxide equivalent compounds, such as GSAO may be prepared by methods known generally in the art.

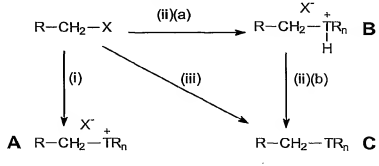
For instance, suitable methods for the synthesis of compounds of formulae (I-VI) and intermediates thereof are described, for example, in Houben-Weyl, *Methoden der Organischen Chemie;* J. March, *Advanced Organic Chemistry*, 4th Edition (John Wiley & Sons, New York, 1992); D. C. Liotta and M. Volmer, eds, *Organic Syntheses Reaction Guide* (John Wiley & Sons, Inc., New York, 1991); R. C. Larock, *Comprehensive Organic Transformations* (VCH, New York, 1989), H. O. House, *Modern Synthetic Reactions* 2nd Edition (W. A. Benjamin, Inc., Menlo Park, 1972); N. S. Simpkins, ed., *100 Modern Reagents* (The Royal Society of Chemistry, London, 1989); A. H. Hains *Methods for the Oxidation of Organic Compounds* (Academic Press, London, 1988) and B. J. Wakefield *Organolithium Methods* (Academic Press, London, 1988).

Example reaction schemes to illustrate the generic formation of linkers of the cell-membrane impermeable arsenoxide compounds are shown in the following schemes.



wherein E represents an electrophilic site; and m, n are integers greater than or equal to 0.

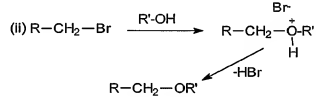
The scheme below shows a starting molecule RCH_2X, where R represents the rest of the molecule to which the $-CH_2X$ group is attached. X represents a leaving group, for example, a halogen or RSO_3-, which is displaced by the nucleophile TR_n. Nucleophiles attack at electrophilic sites, resulting in the formation of a new covalent bond between the nucleophilic and electrophilic species. In the scheme below, the methylene carbon atom is the electrophilic site, and the overall reaction can be described as one of nucleophilic substitution.

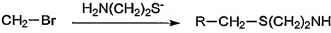


There are three simple variations on the above scheme, as illustrated by reactions (i) to (iii):

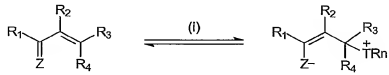
In this reaction, the attacking nucleophile is represented by the uncharged molecule TR_n, which displaces the leaving group X, giving the product **A** which has a positive charge formally localised on T. The first step (a) of this reaction involves the attacking nucleophile HTR_n displacing the leaving group X, giving the ionic product **B** initially, followed by loss of H^+ in step (b) to give the uncharged product **C**. In this reaction, product **C** is formed directly by use of TR_n^- as the nucleophile.

In all three reactions (i) to (iii), X is lost as X^-, and atom T must have a lone pair of electrons. Shown below are general examples of each of the reactions (i) to (iii). Note that reaction (iii) is analogous to the formation of GSAO from BRAO and GSH.

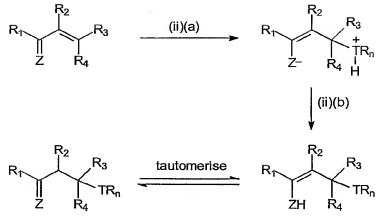




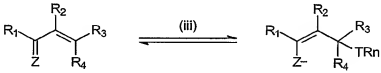
Alternatively, the reaction may be between a nucleophile and, for example, an α,β-unsaturated ketone (when Z=O) (or aldehyde when Z=O and R_1 = H) as illustrated in the following schemes. For example, where the nucleophile is TR_n:



where the nucleophile is HTR_n

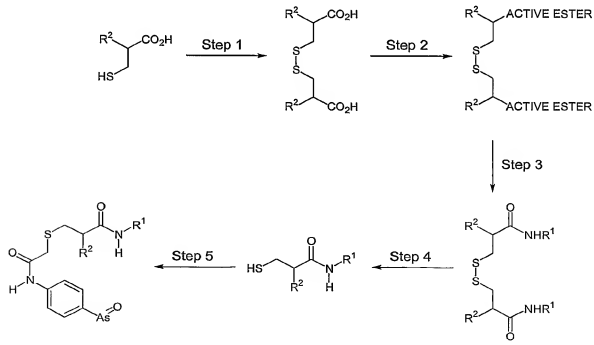


where the nucleophile is TR_n^-.

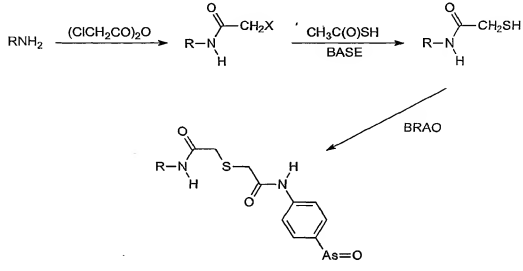


wherein Z is selected from the group consisting of O, S, NR, or +N(R)(R').

An example of a general synthetic route for preparing hydrophilic amine compounds for generating a cell-membrane impermeable arsenoxide compound for use in the invention is represented in the following scheme, wherein the reagent in step 5 has been exemplified as BRAO:



Still more typically, hydrophilic amine compounds can be prepared according to the general scheme outlined below which has been exemplified using BRAO in the final step and wherein X is a halogen or other suitable leaving group.

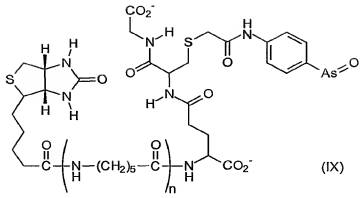


In respect of the above schemes, one skilled in the art would recognise that the various reagents and reactants can be routinely modified in order to synthesise any given compound useful in the invention.

In a typical synthesis of a preferred compound for use in the invention, glutathione may be reacted with BRAO under conditions favourable to the formation of a covalent bond between the free thiol of glutathione and the chemical entity to which the arsenoxide is attached. Reactions involving nucleophilic attack by the glutathione thiol will, in general, require alkaline conditions. Electrophilic attack of some reactive species on the glutathione sulfur atom may be carried out; in general this would likely require acidic conditions.

Suitable modifications will be apparent to those of skill in this art. A person skilled in this art would recognise that the invention also provides for the compounds of the invention in any state of ionisation, for example acid salt, zwitterionic uncharged, zwitterionic anion, dianion.

There is also provided further compounds which are useful in the present invention, wherein these are modified through the glutamyl α-amino nitrogen of glutathione, for example, with a detectable group, such as biotin, a fluorophore, or a group comprising a transition element. For example, the invention provides for GSAO-B, a biotin-linked derivative of GSAO, according to the following formula (IX):

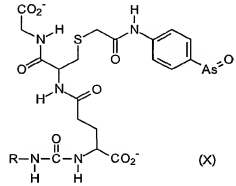


(IX)

wherein n = 1 or 2.

A method of synthesis of GSAO-B is provided in Example 1(a) and illustrated in Figure 7.

An alternative compound for use in the invention in which a desired modifying group may be attached through the glutamyl α-amino nitrogen of glutathione is represented in the following formula (X):



(X)

wherein R is any desired modifying group.

Typically, R may be selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, carboxy, alkoxy, alkyl, and aryl.

Determining Inhibition of Protein Tyrosine Phosphatases

In an exemplary form of the present invention, when determining whether a compound was a modifier of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, the uptake into endothelial cells of a labelled substantially cell-membrane impermeable arsenoxide compound, and subsequent effect on these protein tyrosine phosphatases was monitored. In particular, the compound was GSAO labelled as ^{3}H-GSAO.

Endothelial cells may be incubated with GSAO for a period of time, typically with 10-100 μM concentration for 8 to 72 hours. The cells are then immunostained for phosphotyrosine. For example, incubation with GSAO resulted in contraction of the cells and hyperphosphorylation of tyrosine residues in focal adhesion proteins.

The hyperphosphorylated proteins may be identified by Western blotting and immunoprecipitation. For example, the extent of tyrosine phosphorylation of two proteins with molecular masses of ~120 and ~70 kDa was increased by incubation with GSAO. The molecular masses of these proteins correspond to those of FAK and paxillin. FAK and paxillin were immunoprecipitated from control and GSAO-treated endothelial cells. Treatment with GSAO resulted in ~1.5- and 3-fold increase in tyrosine phosphorylation of FAK and paxillin, respectively.

Tyrosine phosphorylated FAK (FAKpTyr) may be prepared and used as a substrate for PTPases in endothelial cell lysate. For example, dephosphorylation of FAKpTyr was inhibited by GSAO. In addition, endothelial cells may be incubated with GSAO and the lysate tested for PTPase activity using FAKpTyr as the substrate. For example, GSAO-treated cells had ~10% of the FAKpTyr dephosphorylation activity of untreated cells.

The identity of the PTPases that are being inhibited by GSAO in endothelial cells may be determined by incubating endothelial cell lysate with a biotinylated GSAO, immunoprecipitating individual PTPases and blotting with streptavidin-peroxidase to detect incorporation of GSAO. Specific binding can be determined by incubating with GSAO-biotin in the absence or presence of 1 mM 2,3-dimercaptopropanol (DMP). DMP is a small dithiol that competes for binding of GSAO-biotin to protein dithiols. For example, PTP-PEST bound specifically to GSAO-biotin. SHP2 and PTEN did not bind GSAO-biotin.

Therefore, the present invention also relates to processes for the identification of compounds as effective modifiers of protein tyrosine phosphatases selected from the group consisting of: PTP-PEST and PTP-1B, in particular inhibitors of protein tyrosine phosphatases selected from the group consisting of: PTP-PEST and PTP-1B.

Pharmaceutical and/or Therapeutic Formulations

Further, the present invention also relates to compounds (modifiers), in particular, inhibitors which are capable of changing the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and which are preferably detected in accordance with the screening processes set out herein, used alone or together with a pharmaceutically acceptable carrier, adjuvant and/or diluent in methods for the modification of angiogenesis and/or the treatment of angiogenesis dependent disease.

Typically, for medical use, salts of the compounds used in the present invention will be pharmaceutically acceptable salts; although other salts may be used in the preparation of the inventive compound or of the pharmaceutically acceptable salt thereof. By pharmaceutically acceptable salt it is meant those salts which, within the scope of sound medical judgement, are suitable for use in contact with the tissues of humans and lower animals without undue toxicity,

irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art.

For instance, suitable pharmaceutically acceptable salts of the compounds useful in the present invention may be prepared by mixing a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, methanesulfonic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, phosphoric acid, acetic acid, oxalic acid, carbonic acid, tartaric acid, or citric acid with the compounds of the invention. Suitable pharmaceutically acceptable salts of the compounds of the present invention therefore include acid addition salts.

For example, S. M. Berge *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, *66*:1-19. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Representative acid addition salts include acetate, adipate, alginate, ascorbate, asparate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

Also included within the scope of compounds used in the present invention are prodrugs. Typically, prodrugs will be functional derivatives of the compounds of the present invention which are readily converted *in vivo* to the required compounds of the present invention as described herein. Typical procedures for the selection and preparation of prodrugs are known to those of skill in the art and are described, for instance, in H. Bundgaard (Ed), *Design of Prodrugs*, Elsevier, 1985.

Single or multiple administrations of the compounds or pharmaceutical compositions can be carried out with dose levels and pattern being selected by the treating physician. Regardless, the compounds or pharmaceutical compositions used in the present invention should provide a quantity of the compound sufficient to effectively treat the patient.

One skilled in the art would be able, by routine experimentation, to determine an effective, non-toxic amount of the compounds or pharmaceutical compositions used in the invention which would be required to treat or prevent the disorders and diseases. Generally, an effective dosage is expected to be in the range of about 0.0001mg to about 1000mg per kg body weight per 24 hours; typically, about 0.001mg to about 750mg per kg body weight per 24 hours; about 0.01mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 250mg per kg body weight per 24 hours; about 1.0mg to about 250mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range about 1.0mg to about 200mg per kg body weight per 24 hours; about 1.0mg to about 100mg per kg body weight per 24 hours; about 1.0mg to about 50mg per kg body weight per 24 hours; about 1.0mg to about 25mg per kg body weight per 24 hours; about 5.0mg to about 50mg per kg body weight per 24 hours; about 5.0mg to about 20mg per kg body weight per 24 hours; about 5.0mg to about 15mg per kg body weight per 24 hours.

Alternatively, an effective dosage may be up to about 500mg/m^2. Generally, an effective dosage is expected to be in the range of about 25 to about 500mg/m^2, preferably about 25 to about 350mg/m^2, more preferably about 25 to about 300mg/m^2, still more preferably about 25 to about 250mg/m^2, even more preferably about 50 to about 250mg/m^2, and still even more preferably about 75 to about 150mg/m^2.

In relation to GSAO, an effective dosage is in the range of about 0.0001 mg to about 100 mg GSAO per kg body weight per 24 hours, preferably about 0.001 mg to about 100 mg GSAO per kg body weight per 24 hours, more preferably about 0.01 mg to about 50 mg GSAO per kg body weight per 24 hours, even more preferably about 0.1 mg to about 20 mg GSAO per kg body weight per 24 hours, even more preferably still about 0.1 mg to about 10 mg GSAO per kg body weight per 24 hours. Typically the treatment would be for the duration of the condition.

Further, it will be apparent to one of ordinary skill in the art that the optimal quantity and spacing of individual dosages of a compound of the present invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the nature of the particular vertebrate being treated. Also, such optimum conditions can be determined by conventional techniques.

It will also be apparent to one of ordinary skill in the art that the optimal course of treatment, such as, the number of doses of the compound of the present invention given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

Whilst the compounds used in the present invention may be administered alone, it is generally preferable that the compound be administered as a pharmaceutical composition/formulation. In general pharmaceutical formulations of the present invention may be prepared according to methods which are known to those of ordinary skill in the art and accordingly may include a pharmaceutically acceptable carrier, diluent and/or adjuvant.

The carriers, diluents and adjuvants must be "acceptable" in terms of being compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

Examples of pharmaceutically and veterinarily acceptable carriers or diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysolpoxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol; lower aralkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyrridone; agar; carrageenan; gum tragacanth or gum acacia, and petroleum jelly. Typically, the carrier or carriers will form from 10% to 99.9% by weight of the compositions.

In a preferred form the pharmaceutical composition of the invention comprises an effective amount of a compound identified using the methods of the invention, together with a pharmaceutically acceptable carrier, diluent and/or adjuvant as shown in Example 4.

The pharmaceutical compositions of the invention may be administered by standard routes. In general, the compositions may be administered by the topical, transdermal, intraperitoneal, intracranial, intracerebroventricular, intracerebral, intravaginal, intrauterine, oral, rectal or parenteral (e.g., intravenous, intraspinal, subcutaneous or intramuscular) route. Still generally, the compositions of the invention may be in the form of a capsule suitable for oral ingestion, in the form of an ointment, cream or lotion suitable for topical administration, in a form suitable for delivery as an eye drop, in an aerosol form suitable for administration by inhalation, such as by intranasal inhalation or oral inhalation.

The pharmaceutical compositions of the invention may also be administered in the form of liposomes. Liposomes are generally derived from phospholipids or other lipid substances, and are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous

medium. Any non-toxic, physiologically acceptable and metabolisable lipid capable of forming liposomes can be used. The formulations of the present invention in liposome form may contain, in addition to a compound of the present invention, stabilisers, preservatives, excipients and the like. The preferred lipids are the phospholipids and the phosphatidylcholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art, and in relation to this specific reference is made to: Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq.*, the contents of which is incorporated herein by reference.

For administration as an injectable solution or suspension, non-toxic parenterally acceptable diluents or carriers can include, Ringer's solution, isotonic saline, phosphate buffered saline, ethanol and 1,2-propylene glycol.

Some examples of suitable carriers, diluents, excipients and adjuvants for oral use include peanut oil, liquid paraffin, sodium carboxymethylcellulose, methylcellulose, sodium alginate, gum acacia, gum tragacanth, dextrose, sucrose, sorbitol, mannitol, gelatine and lecithin. In addition these oral formulations may contain suitable flavouring and colourings agents. When used in capsule form the capsules may be coated with compounds such as glyceryl monostearate or glyceryl distearate which delay disintegration of the capsule.

Adjuvants typically include emollients, emulsifiers, thickening agents, preservatives, bactericides and buffering agents.

Solid forms for oral administration may contain binders acceptable in human and veterinary pharmaceutical practice, sweeteners, disintegrating agents, diluents, flavourings, coating agents, preservatives, lubricants and/or time delay agents. Suitable binders include gum acacia, gelatine, corn starch, gum tragacanth, sodium alginate, carboxymethylcellulose or polyethylene glycol. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, guar gum, xanthan gum, bentonite, alginic acid or agar. Suitable diluents include lactose, sorbitol, mannitol, dextrose, kaolin, cellulose, calcium carbonate, calcium silicate or dicalcium phosphate. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulfite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

Liquid forms for oral administration may contain, in addition to the above agents, a liquid carrier. Suitable liquid carriers include water, oils such as olive oil, peanut oil, sesame oil,

sunflower oil, safflower oil, arachis oil, coconut oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides, or mixtures thereof.

Suspensions for oral administration may further comprise dispersing agents and/or suspending agents. Suitable suspending agents include sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, poly-vinyl-pyrrolidone, sodium alginate or acetyl alcohol. Suitable dispersing agents include lecithin, polyoxyethylene esters of fatty acids such as stearic acid, polyoxyethylene sorbitol mono- or di-oleate, -stearate or -laurate, polyoxyethylene sorbitan mono- or di-oleate, -stearate or –laurate, and the like.

The emulsions for oral administration may further comprise one or more emulsifying agents. Suitable emulsifying agents include dispersing agents as exemplified above, or natural gums such as guar gum, gum acacia or gum tragacanth.

The topical formulations of the present invention, comprise an active ingredient together with one or more acceptable carriers, and optionally any other therapeutic ingredients.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions. These may be prepared by dissolving the active ingredient in an aqueous solution of a bactericidal and/or fungicidal compound and/or any other suitable preservative, and optionally including a surface active compound. The resulting solution may then be clarified by filtration, transferred to a suitable container and sterilised. Sterilisation may be achieved by: autoclaving or maintaining at 90°C-100°C for half an hour, or by filtration, followed by transfer to a container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those described above in relation to the preparation of drops. Lotions or liniments for application to the skin may also include an compound to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturiser such as glycerol, or oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be prepared by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogols.

The formulation may incorporate any suitable surface active compound such as an anionic, cationic or non-ionic surface active such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

The compositions for parenteral administration will commonly comprise a solution of a compound of the present invention or a cocktail thereof dissolved in an acceptable carrier, such as water, buffered water, 0.4% saline, and 0.3% glycine etc, wherein such solutions are sterile and relatively free of particulate matter.

Methods for preparing parenterally administrable compositions are apparent to those skilled in the art, and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa., hereby incorporated by reference herein.

The pharmaceutical compositions of the invention may also be administered in the form of liposomes. Liposomes are generally derived from phospholipids or other lipid substances, and are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolisable lipid capable of forming liposomes can be used. The formulations of the present invention in liposome form may contain, in addition to a compound of the present invention, stabilisers, preservatives, excipients and the like. The preferred lipids are the phospholipids and the phosphatidylcholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art, and in relation to this specific reference is made to: Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq.*, the contents of which is incorporated herein by reference.

Treatment of Disease

Further, the present invention also relates to the use of compounds (modifiers), in particular, inhibitors of protein tyrosine phosphatases, wherein the protein tyrosine phosphatases are selected from the group consisting of: PTP-PEST and PTP-1B, used alone or in a pharmaceutical formulation, for the treatment of disease.

For instance, the invention relates to a method of inhibiting or stimulating angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier, in the form of an inhibitor or stimulator of these enzymes, or a therapeutically effective amount of a pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

In addition, the invention also relates to methods of inhibiting or stimulating angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier of these protein tyrosine phosphatases, or a pharmaceutical composition comprising at least one modifier. Further, these compounds can be used in the treatment of an angiogenesis dependent disease in a vertebrate.

Further, the invention relates to methods of treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and wherein said method comprises administering a therapeutically effective amount of at least one compound capable of inhibiting the activity of at least one of said protein tyrosine phosphatases.

Thus, the present invention is useful for the treatment of disorders which may be grouped into broad categories such as the following: angiogenesis-dependent diseases, cellular proliferative diseases, inflammatory disorders, auto-immune diseases, blood vessel diseases, thrombosis, viral infection, and cancer.

More typically, the agents capable of changing the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, or pharmaceutical formulations thereof may be useful for the treatment of angiogenesis-dependent diseases, such as cancer, hemangioma, arteriovenous malformations, arthritis, Osler-Weber Syndrome, complicated atherosclerotic plaques, psoriasis, corneal graft neovascularization, pyrogenic granuloma, delayed wound healing, retrolental fibroplasia, diabetic retinopathy, scleroderma, granulations, angiofibroma, neovascular glaucoma, trachoma, hemophilic joints, hypertrophic scars, or gastric ulcers.

Typically, the cancer is selected from the group consisting of carcinogenic tumours, tumours of epithelial origin, such as colo-rectal cancer, breast cancer, lung cancer, head and neck tumours, hepatic cancer, pancreatic cancer, ovarian cancer, gastric cancer, brain cancer, bladder cancer,

prostate cancer and urinary/genital tract cancer; mesenchymal tumours, such as sarcoma; and haemopoietic tumours such as B cell lymphoma.

Typically, the cancer is a haematological tumour. More typically, the cancer is a solid tumour.

The agents capable of changing the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, or pharmaceutical formulations thereof, may also find use in the treatment of human retroviral infections (family retroviridae) including, for example, oncoviral infection such as HTLV-I; Lentiviral infection including HIV-1 and HIV-2; or for the treatment or prevention of Sindbis virus infection.

The compounds capable of changing the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, or pharmaceutical formulations thereof, may also be used in the treatment of inflammatory disorders and/or auto-immune diseases, examples of which include the following: rheumatoid arthritis, seronegative arthritides and other inflammatory arthritides, systemic lupus erythematosus, polyarteritis and related syndromes, systemic sclerosis, Sjögren's syndrome and other inflammatory eye disease, mixed connective tissue disease, polymyositis and dermatomyositis, polymyalgia rheumatica and giant cell arteritis, inflammatory joint disease, non-inflammatory arthropathies and soft tissue rheumatism, algodystrophy.

Examples of vascular disease and thrombosis for which these compounds or pharmaceutical formulations thereof may be used in the treatment of, include the following: progression of atherosclerosis; cerebrovascular accidents such as transient ischaemic, completed stroke, and after carotid surgery; acute myocardial infarction (primary and secondary); angina; occlusion of coronary artery bypass graft; occlusion following percutaneous transluminal coronary angioplasty; occlusion following coronary stenting; vascular occlusion in peripheral arterial disease; venous thromboembolic disease following surgery, or during pregnancy, or during immobilisation.

Examples of small vessel disease for which these compounds or pharmaceutical formulation thereof may be used in treatment of, include the following: glomerulonephritis; thrombotic thrombocytopenic purpura; the haemolytic uraemic syndrome; placental insufficiency and preeclampsia.

Those agents capable of changing the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B may also find use in the prevention of thrombosis formation in the following situations: artificial/prosthetic vascular shunts and grafts; prosthetic heart valves; cardiopulmonary bypass procedures; haemoperfusion and haemodialysis.

Typically, the compounds used in the invention may be used in combination with other known treatments, such as surgery and/or therapeutic agents, including chemotherapeutic or

radiotherapeutics. For example, when used in the treatment of solid tumours, these compounds may be administered with chemotherapeutic agents such as: adriamycin, taxol, fluorouricil, melphalan, cisplatin, alpha interferon, COMP (cyclophosphamide, vincristine, methotrexate and prednisone), etoposide, mBACOD (methortrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone), PROMACE/MOPP (prednisone, methotrexate (w/leucovin rescue), doxorubicin, cyclophosphamide, taxol, etoposide/mechlorethamine, vincristine, prednisone and procarbazine), vincristine, vinblastine, angioinhibins, TNP-470, pentosan polysulfate, platelet factor 4, angiostatin, LM-609, SU-101, CM-101, Techgalan, thalidomide, SP-PG and the like. Other chemotherapeutic agents include alkylating agents such as nitrogen mustards including mechloethamine, melphan, chlorambucil, cyclophosphamide and ifosfamide; nitrosoureas including carmustine, lomustine, semustine and streptozocin; alkyl sulfonates including busulfan; triazines including dacarbazine; ethyenimines including thiotepa and hexamethylmelamine; folic acid analogues including methotrexate; pyrimidine analogues including 5-fluorouracil, cytosine arabinoside; purine analogues including 6-mercaptopurine and 6-thioguanine; antitumour antibiotics including actinomycin D; the anthracyclines including doxorubicin, bleomycin, mitomycin C and methramycin; hormones and hormone antagonists including tamoxifen and cortiosteroids and miscellaneous agents including cisplatin and brequinar.

Typically, the vertebrate is selected from the group consisting of human, non-human primate, murine, bovine, ovine, equine, caprine, leporine, avian, feline and canine. More typically, the vertebrate is human, non-human primate or murine. Even more typically, the vertebrate is human.

Diagnosis of Disease

The present invention also provides a method of diagnosing an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and wherein said method comprises administering a diagnostically effective amount of at least one inhibitor of these enzymes, and assessing clinical improvement in signs and symptoms of said disease in said vertebrate.

Given that the inhibitors of protein tyrosine phosphatases of the invention, either administered alone or in the form of a composition, are safe when administered in accordance with the methods of the invention, then therapeutic challenge with at least one protein tyrosine phosphatase of the invention is an important diagnostic tool for suspected angiogenesis dependent disease, especially when considered together with other aspects of angiogenesis dependent disease.

The examples set out below, in particular, Examples 2(f)-2(i) relating to clinical treatment of angiogenesis dependent disease exemplify the feasibility of diagnosing an angiogenesis dependent disorders using inhibitors of protein tyrosine phosphatases.

The invention will now be described in greater detail by reference to specific Examples which should not be construed as in any way limiting the scope of the invention.

Examples

Example 1: Experimental Procedures

Example 1(a): Synthesis of GSAO, GSAO-biotin and GSAA

GSAO, GSAO-biotin and GSAA were synthesized, characterized and purified as described in International (PCT) Patent Application Number PCT/AU00/01143 (WO 01/21628) the disclosure of which is incorporated herein by reference. The synthesis of Synthesis of 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide (GSAO) is as follows and is also represented schematically in Figure 8.

Synthesis of 4-(N-(bromoacetyl)amino)phenylarsonic acid (BRAA)

Sodium carbonate (40.14 g, 378.7 mmol) was added to water (200 mL) and stirred at room temperature until all solids had dissolved. To the stirred carbonate solution was added p-arsanilic acid (29.99 g, 138.2 mmol), portionwise, and the volume of the solution made up to 300 mL with addition of more water. The solution (pH 10 to 11) was allowed to stir for 30 mins, and if necessary, was filtered to remove any undissolved solid before being refrigerated for 2 to 3 hours. The solution was transferred to a separating funnel and ice chips were added. Bromoacetyl bromide (15 mL, 34.76 g, 172.1 mmol) was diluted in dichloromethane (50 mL) and approximately half of the dichloromethane solution was added carefully to the cold aqueous solution. The mixture was cautiously shaken, with frequent venting to avoid excessive build up of pressure. After 1 to 2 mins, the evolution of carbon dioxide had subsided, and more vigorous shaking was undertaken. The remaining portion of bromoacetyl bromide was carefully added and the procedure repeated. When the reaction was over, the solution was found to be pH 7. The lower dichloromethane layer was discarded, and the aqueous layer transferred to a 1 L flask and carefully acidified by dropwise addition of 98% sulfuric acid. Complete precipitation of the white product required addition of acid until the solution was approximately pH 1. The crude product was collected and dried at the pump, typically in yields of 50% to 75%. ^{1}H-NMR (d_6-DMSO): δ 4.09 (s, 2H), 7.73 (d, J = 9 Hz, 2H), 7.83

(d, J = 9 Hz, 2H), 10.87 (s, 1H). ^{13}C-NMR (d_6-DMSO): δ 30.53, 119.97, 127.34, 131.56, 143.08, 166.00 ppm.

Synthesis of 4-(N-(bromoacetyl)amino)phenylarsenoxide hydrate (BRAO.xH_2O)

Into a 3-necked 500 mL round-bottomed flask was placed BRAA (12.15 g, 36 mmol). The solid was dissolved with swirling in a mixture of methanol (75 mL) and hydrobromic acid (48%, 75 mL), giving a transparent yellow solution. The solution was filtered to remove residual solids. Sodium iodide (0.20 g, 1.3 mmol) was added as a catalyst, whereupon the colour of the solution darkened to orange-brown, then sulfur dioxide gas was slowly (ca. 2 bubbles per second) passed through the stirred solution for approximately 2.5 hours. The resultant white precipitate was collected using a Büchner funnel, giving the product (17.43 g) as a damp white solid. The activity of a solution made by dissolving a portion of the solid (40.7 mg) in deoxygenated DMSO (800 µL) was determined to be 56 mM (see below). Hence, the molecular weight of BRAO.xH_2O is 908.5, that is, 35% w/w BRAO and 65% w/w H_2O. Therefore, the "anhydrous" weight of the BRAO product was 35% of 17.43 g, that is, 6.10 g (19 mmol, 53% yield). ^{1}H-NMR (d_6-DMSO): δ 4.85 (s, 2H), 7.78 (d, J = 9 Hz, 2H), 7.86 (d, J = 9 Hz, 2H), 11.36 (s, 1H). ^{13}C-NMR (d_6-DMSO): δ 30.55, 119.22, 130.52, 140.04, 145.04, 165.52 ppm.

Synthesis of 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide (GSAO)

DMSO (10 mL) was deoxygenated by passing a stream of nitrogen gas through it for a few minutes, and used to dissolve BRAO.xH_2O (1.00 g, 2.48 mmol active arsenoxide). Glutathione (1.15 g, 3.74 mmol, 1.5 eq) was dissolved in 0.5 M bicarbonate buffer, pH 9.6 (35 mL), and added to the solution of BRAO.xH_2O in DMSO. The total volume was made up to 50 mL with 0.5 M bicarbonate buffer, and the solution gently agitated at room temperature overnight. Cautious neutralisation with 37% hydrochloric acid, followed by lyophilisation gave a white powdery product, which could be dissolved in water leaving no residual solid. The active arsenoxide concentration of the resultant solution was found to be 49.6 mM.

The product was purified using gel-filtration (P-2 Gel extra fine, 1.8 kDa cutoff, 50 g) on a 130 mL column, using 20 mM Hepes, 0.14 M NaCl, 1 mM EDTA, pH 7.4 buffer as the eluant at a flow rate of 0.10 mL/min. A total of 144 mL was collected (72 fractions of 2 mL) and monitored by UV (λ 214nm). Four peaks, A, B, C and D, were resolved. Peaks B and C showed activity in the DTNB/DMP assay (see below), and were assigned as GSAO and unreacted BRAO, respectively. Peaks A and D were tentatively assigned as the oxidation products GSAA and BRAA (the oxidation product of BRAO), respectively. Unreacted glutathione was also detected (using DTNB) in the fractions corresponding to Peak A. The fractions corresponding to peak B were combined and

deoxygenated with nitrogen gas to give a solution of GSAO (15 mM, approximately 12 mL). ^{1}H-NMR (D_2O): δ 1.93 (q, J = 7 Hz, 2H), 2.35 (t, J = 8 Hz, 2H), 2.84 (dd, J = 14 Hz, J = 9 Hz, 1H), 3.05 (dd, J = 14 Hz, J = 5 Hz, 1H), 3.35 (s, 2H), 3.58 (t, J = 6 Hz, 1H), 3.64 (d, J = 2 Hz, 2H), 4.48 (dd, J = 9 Hz, J = 5 Hz, 1H), 7.44 (d, J = 8 Hz, 2H), 7.58 (d, J = 8 Hz, 2H). ^{13}C-NMR (D_2O): δ 25.93, 31.16, 33.53, 36.01, 42.97, 52.83, 53.89, 121.29, 129.97, 138.77, 144.09, 170.90, 171.73, 173.75, 174.68, 175.76 ppm.

Synthesis of 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsonic acid (GSAA)

The synthesis of GSAA is represented schematically in Figure 9.

BRAA (1.00 g, 2.96 mmol) and glutathione (1.36 g, 4.44 mmol, 1.5 eq) were dissolved in 0.5 M bicarbonate buffer, pH 9.6 (50 mL), and the solution gently agitated at room temperature overnight. Lyophilisation gave a white powdery product which was freely soluble in water, leaving no solid residue. The product was purified by gel-filtration on a 570 mL column (2.5 x 117 cm) of Bio-Gel P-2 extra fine (BioRad, Hercules, CA) using deionised water as the eluant at a flow rate of 0.1 mL per min. The product (GSAA) eluted from the column in a position corresponding to Peak A in the purification of GSAO.

Synthesis of 4-(*N*-(*S*-(*N*-(6-((6-((biotinoyl)amino)hexanoyl)amino)hexanoyl)glutathionyl)acetyl)-amino)phenylarsenoxide (GSAO-B)

The synthesis of GSAO-B is represented schematically in Figure 7.

GSAO (0.13 g) was dissolved in 0.5 M sodium bicarbonate buffer (5 mL, pH 8.5) and the concentration of active arsenical in the resultant solution was determined to be 39 mM. The buffered arsenical solution (4.2 mL, containing 165 μmol active arsenical) was added to a solution of biotin-XX, SE (100 mg, 176 μmol) in DMSO (1 mL), the mixture inverted a few times and then incubated at 4°C for 4 hours. Glycine (17.5 mg, 233 μmol) was added and the mixture kept at 4°C overnight. The concentration of trivalent arsenical in the GSAO-B product was determined to be 31 mM and the solution was used without further modification.

Storage

The arsenicals were reconstituted to concentrations of ~50 mM in phosphate buffered saline (PBS) containing 100 mM glycine, sterile filtered and stored at 4°C in the dark until use. The glycine slows the oxidation of GSAO to GSAA. The active concentration of GSAO was measured by titrating the GSAO with 2,3-dimercaptopropanol and measuring the remaining free thiols with 5,5'-dithiobis(2-nitrobenzoic acid) (Donoghue et al., 2000). There was no significant loss in the active concentration of stock solutions of GSAO for at least 6 weeks when stored under these conditions.

Example 1(b): Synthesis of ^{3}H-GSAO and GSCA

^{3}H-GSAO was prepared as described for GSAO using glycine-2-^{3}H-glutathione (NEN, Boston, MA) in place of unlabelled glutathione.

GSCA was prepared by coupling 4-(N-(bromoacetyl)amino)benzoic acid to reduced glutathione. 4-Aminobenzoic acid (13.04 g, 95.1 mmol) was added in portions to a solution of sodium carbonate (20.32 g, 191.7 mmol) in water (200 mL). When all solids had dissolved, the solution was found to be pH 10, and was chilled at 4°C for 2 h. Bromoacetyl bromide (15 mL, 172.5 mmol) in dry dichloromethane (35 mL) was added in two portions, each addition followed by vigorous shaking for 2 to 3 min. When all shaking had finished, the product began to precipitate out of solution (pH 5) and was collected and dried. 4-(N-(bromoacetyl)amino)benzoic acid (2.70 g, 10.11 mmol), reduced glutathione (3.23 g, 10.51 mmol) and sodium bicarbonate (3.15 g, 37.50 mmol) were mixed together, and the solid mixture was dissolved in portions in 0.5 M bicarbonate buffer (100 mL). The clear solution was found to be pH 9, and was thoroughly mixed and left overnight at room temperature. On the following day, the solution was acidified to neutral pH with 32% hydrochloric acid, and the product precipitated from absolute ethanol (1 L) by dropwise addition to the well-stirred alcohol. The mixture was stirred at room temperature for 1 hour, and then left for 3 hours until the precipitate had settled. The clear ethanolic solution was decanted until ~300 mL were left, and then this was swirled and centrifuged at 2000g for 5 min. The product was washed by re-suspending in fresh absolute ethanol and centrifuged again. The washing was repeated two more times, and the final suspension was dried to a white solid by rotary evaporation.

Example 1(c): Antibodies

Monoclonal antibodies that recognize phosphotyrosine (PY20), paxillin, PTP-1B, SHP2 and PTEN were obtained from Transduction Laboratories, Lexington, KY. Rabbit anti-FAK and peroxidase-coupled anti-phosphotyrosine (PT66) polyclonal antibodies were from Sigma, St. Louis, MO. Rabbit polyclonal antiserum that recognizes human PTP-PEST was a gift from Michael Schaller (Shen et al., 1998). Peroxidase-coupled anti-mouse and anti-rabbit IgG and fluorescein-coupled anti-mouse IgG were obtained from Dako, Carpinteria, CA. Peroxidase-coupled avidin was from Molecular Probes, Eugene, OR.

Example 1(d): Cell Culture

BCE (Folkman et al., 1979), BAE (Hotchkiss et al., 1998), HMEC-1 (Ades et al., 1992), BVSM (Hogg et al., 1997), and BxPC-3, AsPC-1, HT1080 and 3T3 cells (ATCC, Bethesda, MD) were harvested and cultured as indicated. Culture plates were from Corning Costar, Corning, NY. Dulbecco's Modified Eagle's Medium (DMEM) was from JHR Bioscience, Lenexa, KS; MCDB-131

medium, epidermal growth factor and trypsin/EDTA from Gibco BRL, Gaithersburg, MD; fetal calf serum (FCS) from Intergen, Purchase, NY; bovine calf serum (BCS) from HyClone, Logan, UT; Glutamine Pen-Strep from Irvine Scientific, Santa Ana, VA; FGF-2 from Genzyme, Cambridge, MA; VEGF from Genzyme, Cambridge, MA; and hydrocortisone from Sigma, St. Louis, MO.

Adherent cells were counted electronically or stained with methylene blue and counted. Cells were dispersed in trypsin/EDTA, resuspended in Coulter balanced electrolyte solution and counted with a Z1 Coulter counter (Coulter Corp., Miami, FL). Methylene blue absorbances were converted to cell numbers using a standard curve of absorbance plotted against attached cells for each cell type (Oliver et al., 1989).

Example 1(e): Western Blotting

Protein samples were electrophoresed on 8-16% gradient SDS-polyacrylamide gels (SDS-PAGE) (Gradipore, Sydney) and transferred to polyvinyldiethylene fluoride (Millipore, Bedford, MA). PBS containing 0.05% Tween 20 and 5% skim milk powder was used for blocking of all membranes except for those to be probed with PT66-peroxidase, which were blocked with PBS/Tween 20 containing 1% BSA. PT66-peroxidase was diluted 1:5000, while avidin-peroxidase and anti-paxillin and anti-FAK antibodies were diluted 1:2000. Blots were developed using ECL enhanced chemiluminescence (NEN, Boston, MA). Densitometry was performed with Molecular Dynamics ImageQuant v3.3 software.

Example 1(f): Immunoprecipitation

FAK and paxillin were immunoprecipitated from cell lysates in RIPA buffer (10 mM Tris pH 7.4, 150 mM NaCl, 1 mM EGTA, 1% (v/v) Triton X-100, 1% (w/v) sodium deoxycholate, 0.1% SDS, 50 $\mu g.mL^{-1}$ 4-(2-aminoethyl)-benzenesulfonylfluoride, 10 $\mu g.mL^{-1}$ leupeptin, 1 μM aprotinin, 1 $\mu g.mL^{-1}$ pepstatin A). PTPases were immunoprecipitated from cell lysates in 1% Triton X lysis buffer (10 mM Tris pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% (v/v) Triton X-100, 50 $\mu g.mL^{-1}$ 4-(2-aminoethyl)-benzenesulfonylfluoride, 10 $\mu g.mL^{-1}$ leupeptin, 1 μM aprotinin, 1 $\mu g.mL^{-1}$ pepstatin A). Proteins were immunoprecipitated from the lysate by adding 10 $\mu g.mL^{-1}$ antibody and 1:20 (v/v) Protein-G Sepharose (Amersham Pharmacia, Uppsala, Sweden), and incubating the lysate for 4 h at 4°C with agitation. The beads were then washed twice with lysis buffer, resuspended in SDS-PAGE buffer and stored at –20°C until use.

Example 1(g): Cell Treatments for Phosphotyrosine Blotting and Immunoprecipitation

Treated cells were washed twice with PBS and lysed for 30 min at 4 °C in RIPA buffer. 1 mM Na_3VO_4 (Sigma, St. Louis, MO) was added from a 100 mM solution prepared immediately prior to use. The lysates were clarified at 14000 rpm for 5 min and stored at –20°C until use.

Example 1(h): *In vitro* Tyrosine Phosphatase Assay

Phosphorylated FAK was prepared by treating ~10^7 HMEC-1 with 10 μM PAO for 30 min. Cells were lysed in 1 ml RIPA buffer for 30 min at 4 °C, scraped into an eppendorf tube and the lysate was clarified at 14000 rpm for 5 min. FAK was immunoprecipitated as described above, aliquoted and stored at –20°C until use. To assess the affect of GSAO on phosphatase activity in whole-cell lysates, 50 μg HMEC-1 lysate in 1% Triton X lysis buffer was pre-incubated with GSAO for 10 min at 37°C. The lysate was then added to the FAK immunoprecipitates and incubated for a further 90 min at 37°C with agitation. The reaction tubes were then transferred to ice, washed twice with lysis buffer, resuspended in SDS-PAGE buffer and stored at –20°C until use. To asses the phosphatase activity in lysates of GSAO-treated cells, 5 x 10^5 HMEC-1 in a 6-well plate were treated with GSAO, washed twice with PBS and lysed in 1% Triton X lysis buffer. FAK immunoprecipitates were incubated with 50 μg lysate for 90 min at 37°C with agitation.

Example 1(i): Immunofluorescence Microscopy

Treated cells were washed twice with PBS, fixed for 10 min with 3.7% formaldehyde in PBS, permeabilized for 5 min with 3.7% formaldehyde/0.1% Triton X-100 in PBS, then washed twice for 15 min with PBS containing 1% BSA. Fixed cells were incubated overnight at 4°C with a 1:100 dilution of mouse anti-phosphotyrosine antibody, washed twice and incubated for 1 h at room temperature with a mixture of 1:20 fluorescein-conjugated anti-mouse-IgG antibody. The stained cells were washed three times with PBS and mounted in VectaShield antifade agent (Vector Laboratories, Burlingame, CA). Micrographs were taken at 600× magnification using an Olympus BX60 microscope with BX-FLA fluorescence, and a Diagnostic Instruments Spot Digital Camera and v 2.2 software (Sterling Heights, MI).

Example 1(j): Uptake of ^{3}H-GSAO

5 x 10^5 HMEC-1 were incubated with ^{3}H-GSAO, washed twice with PBS, detached with 20 mM EDTA/PBS, washed once with PBS and resuspended in 100 μl PBS containing proteinase inhibitors (50 μg.mL^{-1} 4-(2-aminoethyl)-benzenesulfonylfluoride, 10 μg.mL^{-1} leupeptin, 1 μM aprotinin, 1 μg.mL^{-1} pepstatin A). The cells were lysed by three freeze-thaw cycles and centrifuged at 20800 g for 30 min at 4°C. The supernatant cytosolic fraction was mixed with 5 mL aqueous scintillant ACSII (Amersham Pharmacia, Uppsala, Sweden) and counted.

Example 1(k): CAM Assay

Fertilized 3 day-old white Leghorn eggs (Spafas, Norwich, CT) were cracked, the embryos with intact yolks placed in 20 x 100 mm petri dishes and incubated for 3 days at 37°C and 3% CO_2 (Folkman, 1985). Methylcellulose (Fisher Scientific, Fair Lawn, NJ) discs containing 5, 10 or 50 μg of

either GSAA or GSAO were then applied to the CAM of individual embryos and incubated for 48 h at 37°C and 3% CO_2. The discs were made by desiccation of GSAA or GSAO in 10 µl of 0.45% methycellulose on teflon rods. The CAMs were observed using a stereomicroscope and scored for no obvious affect or inhibition of CAM angiogenesis as defined by avascular zones. On some occasions CAM blood vessels were injected with India ink and photographed.

Example 1(i): Primary Tumor Growth Assays

Female 7 to 9 week old SCID or C57Bl6/J mice were used (Massachusetts General Hospital, Boston, MA). Mice were held in groups of 3 to 5 at a 12 hour day and night cycle and were given animal chow and water *ad libidum*. Mice were anesthetized by inhalation of isoflurane, the dorsal skin shaved and cleaned with ethanol, and a suspension of 2.5 x 10^6 BxPC-3, AsPc-1, HT1080 or LLC cells in 0.2 mL of PBS or saline for LLC cells was injected *S.C.* in the proximal midline. LLC cells were prepared according to O'Reilly et al. (1994). Tumor volume was calculated using the relationship, $a.b^2.0.52$, where a is the longest and b the shortest diameter.

Example 1(i): Immunohistochemistry

Tumors were fixed in Buffered Formalde-Fresh (Fisher Scientific, Fair Lawn, NJ), embedded in paraffin and 5 µm thick sections were cut and placed on glass slides. Sections were stained with haematoxylin and eosin or for CD31, PCNA (Holmgren et al. 1995) or fragmented DNA (Gavrielli et al., 1992). Microvessels were counted in 3 tumors, including the smallest and largest, from the control and treatment groups and the density was graded in the most active areas of neovascularisation according to Weidner et al. (1991). The proliferative index was estimated by the percentage of cells scored under 400× magnification. A minimum of 1000 cells was counted in two separate sections. The apoptotic index was estimated by the percentage of cells scored under 400x magnification. A minimum of 1500 cells was counted in two separate sections.

Example 2: Results

Example 2(a): GSAO Slowly Entered Endothelial Cells Resulting in Cell Detachment

The structures of PAO and GSAO are shown in Figure 1A. Coupling PAO to reduced glutathione decreased the cytotoxicity for endothelial cells by ~2,000-fold. Adherent bovine aortic endothelial (BAE) cells were incubated with increasing concentrations of PAO, GSAO or GSAA for 24 h and the number of adherent cells remaining was measured (Figure 1B). The IC_{50} for cell viability was ~25 nM for PAO and ~50 µM for GSAO. The control GSAA did not significantly affect BAE viability up to a concentration of 0.5 mM. The same result was observed using transformed human dermal microvascular endothelial cells (HMEC-1) or primary human umbilical vein endothelial cells (not shown).

In the present invention the interaction of GSAO with closely-spaced dithiols of certain proteins on the endothelial cell-surface and the affects of GSAO on cell viability by blocking endothelial cell-surface thiols with either 3-(N-maleimidylpropionyl)biocytin (MPB) or 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) prior to incubation with GSAO was considered. The maleimide moiety of MPB interacts rapidly (second order rate constant of ~10^4 $M^{-1}s^{-1}$ at neutral pH) and irreversibly with accessible protein thiols, whilst DTNB interacts rapidly but reversibly with protein thiols (Jiang et al., 1999). GSAO interacts with a subset of the MPB or DTNB-labeled proteins (Donoghue et al., 2000). Adherent HMEC-1 were incubated with either 10 or 100 μM MPB or DTNB prior to addition of GSAO and cultured for 24 h. The number of adherent cells remaining was measured (Figure 1C). Pre-treatment with either MPB or DTNB did not significantly change the affect of GSAO on cell viability.

The time-dependence of the affect of GSAO on endothelial cell viability was then determined. Adherent bovine capillary endothelial (BCE) cells were incubated with either 10 μM GSAO or GSAA and cultured for discrete times up to 48 h. The number of adherent cells remaining was measured (Figure 1D). There was no affect of GSAO on cell viability at 8 h but cell number decreased thereafter. GSAA had no affect on proliferation or viability. This result suggested that GSAO was accumulating in the endothelial cells and affecting viability once a threshold concentration was achieved.

This theory was tested by measuring the kinetics of uptake of ^{3}H-GSAO into endothelial cells. Adherent HMEC-1 cells were incubated with 3 μM ^{3}H-GSAO for 1, 2 or 3 days. The cells were lysed and the cytosolic levels of ^{3}H-GSAO were determined (Figure 1E). There was a linear accumulation of ^{3}H-GSAO in the cytosol of the endothelial cells. A similar result was observed using BAE cells (not shown). Assuming a BAE cell volume of 1.2 pL (Behmanesh and Kempski, 2000), the concentration of ^{3}H-GSAO in the cytosol of the cells after 3 days was ~80 nM.

Example 2(b): GSAO Inhibited Dephosphorylation of Focal Adhesion Kinase (FAK) and Paxillin in Endothelial Cells

The consequence of uptake of GSAO into endothelial cells for morphology and tyrosine phosphorylation of cell proteins was measured by immunofluorescence microscopy. Adherent HMEC-1 were incubated without or with 100 μM GSAO for 10 h. The cells were washed and immunostained for phosphotyrosine (Figure 2A). Incubation with GSAO resulted in contraction of the cells and hyperphosphorylation of tyrosine residues in focal adhesion proteins. The association of tyrosine phosphorylated proteins with focal adhesions was confirmed by comparing Figure 2A

with GSAO-treated HMEC-1 stained for paxillin or the actin cytoskeleton (not shown). The identity of the hyperphosphorylated proteins was determined by Western blotting and immunoprecipitation.

Adherent HMEC-1 were incubated with GSAO or PAO and samples of the cells were resolved on SDS-PAGE, transferred to PVDF membrane and blotted for phosphotyrosine. The extent of tyrosine phosphorylation of two proteins with molecular masses of ~120 and ~70 kDa was changed by incubation with GSAO (Figure 2B). The molecular masses of these proteins correspond to those of FAK and paxillin. Incubation of HMEC-1 with GSAA did not change the pattern or intensity of tyrosine phosphorylated proteins (not shown).

FAK and paxillin were immunoprecipitated from control and GSAO-treated HMEC-1. Treatment with GSAO resulted in elevated tyrosine phosphorylation of both and FAK and paxillin (Figure 2C). This affect of GSAO was both time- and concentration-dependent (Figure 2B). Treatment with 100 μM GSAO for 8 h resulted in ~1.5- and 3-fold increase in tyrosine phosphorylation of FAK and paxillin, respectively.

Example 2(c): GSAO Inhibited Dephosphorylation of FAK and Bound to PTP-PEST and PTP-1B in Endothelial Cell Lysate

Tyrosine phosphorylated FAK (FAKpTyr) was prepared in HMEC-1 by incubation with PAO and collected by immunoprecipitation. The FAKpTyr was used as a substrate for PTPases in endothelial cell lysate. Inhibition of dephosphorylation of FAKpTyr by vandate, PAO or GSAO was measured (Figure 2D). As anticipated, both vanadate and PAO inhibited FAKpTyr dephosphorylation. GSAO also inhibited FAKpTyr dephosphorylation and the inhibition was concentration-dependent. Incubation with 2 μM GSAO resulted in ~50% inhibition of PTPase activity. Similar results were obtained using phosphorylated paxillin in place of FAKpTyr (not shown).

It was next determined whether incubation of intact endothelial cells with GSAO would result in inhibition of FAKpTyr phosphatase(s). HMEC-1 were incubated in the absence or presence of 100 μM GSAO for 8 h and the lysate tested for PTPase activity using FAKpTyr as the substrate. GSAO-treated cells had ~10% of the FAKpTyr dephosphorylation activity of untreated cells (Figure 2E).

To identify which of the PTPases were interacting with GSAO in endothelial cells, a biotin moiety was attached through a spacer arm to the primary amino group of the γ-glutamyl residue of GSAO to produce (4-(*N*-(*S*-(*N*-(6-((6-((biotinoyl)amino)hexanoyl)amino)hexanoyl)-glutathionyl)acetyl)amino)phenylarsenoxide (GSAO-biotin). Binding of GSAO-biotin to PTPases was measured by blotting the biotin with streptavidin-peroxidase. Biotinylation of GSAO does not affect its binding to closely-spaced protein thiols (Donoghue et al., 2000). Lysate of HMEC-1 was

incubated with 2 μM GSAO-biotin in the absence or presence of 1 mM 2,3-dimercaptopropanol (DMP). DMP is a small dithiol that competes for binding of GSAO-biotin to protein dithiols. The dissociation constant for binding of DMP to GSAO is ~130 nM (Donoghue et al., 2000). PTP-PEST, SHP2 or PTEN were immunoprecipitated from the lysate and blotted with streptavidin-peroxidase (Figure 2F). PTP-PEST bound specifically to GSAO-biotin. SHP2 and PTEN did not bind GSAO-biotin.

PTP-1B is a cytosolic PTPase that interacts with and dephosphorylates $p130^{Cas}$ (Liu et al, 1996). HMEC-1 lysate was incubated with GSAO-biotin in the absence or presence of DMP and the PTP-1B was immunoprecipitated and analyzed as described above. PTP-1B bound specifically to GSAO-biotin (Figure 2F).

Example 2(d): GSAO was a Selective Inhibitor of Endothelial Cell Proliferation and Viability

GSAO inhibited the proliferation and reduced the viability of BCE cells in a 72 h proliferation assay (Figures 3A and 3B). In contrast, GSAA had only a marginal affect on proliferation/viability at the highest concentration used, 1 mM. Inhibition of proliferation by GSAO was independent of whether the cells were stimulated with either fibroblast growth factor-2 (FGF-2) (Figure 3A) or vascular endothelial cell growth factor (VEGF) (Figure 3B). The IC_{50} for inhibition of proliferation in response to FGF-2 or VEGF was ~0.1 μM and ~0.05 μM, respectively (Table 1). GSAO also reduced the viability of BCE cells with IC_{50} values of ~0.4 μM and ~2 μM in the presence of FGF-2 or VEGF, respectively (Table 1).

GSAO inhibited proliferation and reduced viability of BAE cells with IC_{50} values in the same range as for BCE cells (not shown). GSAA did not affect either proliferation or viability. It is noteworthy that the IC_{50} for inhibition of proliferation of BAE cells was ~0.02 μM when 5% FCS was the mitogen versus ~0.2 μM when 10% FCS was used (Table 1).

High micromolar concentrations of GSAO were required to inhibit the proliferation and viability of the human tumor cell lines, BxPC-3 (Figure 3C) or HT1080 (not shown), murine 3T3 fibroblasts (not shown) and bovine vascular smooth muscle (BVSM) cells (not shown). IC_{50} values for proliferation and viability were between 15 and 40 μM (Table 1). GSAA had no significant affect on proliferation or viability of these cells up to a concentration of 1 mM.

Table 1. Effects of GSAO on proliferation and viability of cultured cells in a 72 hour proliferation assay.

Cell	Mitogen	Proliferation IC_{50}, μM[a]	Viability IC_{50}, μM[b]
BCE	5% BCS + 1 ng.mL^{-1} FGF-2	~0.1	~0.4
BCE	5% BCS + 10 ng.mL^{-1} VEGF	~0.05	~2
BAE	5% BCS	~0.02	~4
BAE	10% BCS	~0.2	~3
BxPC-3	5% FCS	~20	~40
HT1080	5% FCS	~15	~40
BVSM	5% BCS	~20	~40
3T3	5% FCS	~20	~40

a The IC_{50} for inhibition of proliferation of cells was calculated as the concentration of GSAO that reduced the extent of proliferation by half in a 72 hr incubation.

b The IC_{50} for reduction in viability of cells was calculated as the concentration of GSAO that reduced the cell number by half in a 72 hr incubation.

Example 2(e): GSAO was Toxic for Proliferating but not Growth Quiescent Endothelial Cells

Adherent BAE cells were induced into growth quiescence by incubating with 0.25% FCS for 48 h and the medium was then replaced with either 0.25% or 10% FCS containing either 50 μM GSAO or 0.5 μM PAO. The number of adherent cells after 48 h was measured.

GSAO did not significantly affect the viability of growth quiescent endothelial cells but reduced the viability of proliferating endothelial cells by >90%. In contrast, PAO at a 100-fold lower concentration than GSAO reduced the viability of both growth quiescent and proliferating endothelial cells equally. The rate of uptake of ^{3}H-GSAO into growth quiescent BAE cells (not shown) was similar to that for proliferating BAE cells (see Figure 1E), which implied that the increased toxicity of GSAO for proliferating BAE cells was not a result of increased uptake of GSAO.

These findings indicated that GSAO was a selective inhibitor of proliferating microvascular and macrovascular endothelial cells.

Example 2(f): GSAO Inhibited CAM Angiogenesis

The chick CAM assay has been used for the detection and analysis of angiogenesis inhibition (Nguyen et al., 1994). GSAO inhibited CAM angiogenesis in a concentration-dependent manner (Figure 4A).

Angiogenesis inhibition was defined as avascular zones 48 h after implantation of methylcellulose pellets containing GSAO on the 6-day CAM (Figure 4B). GSAA up to 50 μg per pellet had no affects on CAM angiogenesis.

Example 2(g): GSAO Inhibited Human and Murine Primary Tumor Growth

The growth of both human and murine primary tumors in immunocompromised and immunocompetent mice, respectively, was markedly suppressed by systemic administration of GSAO.

S.C. administration of 2 mg/kg/day GSAO resulted in ~70% inhibition of the rate of BxPC-3 tumor growth in SCID mice (Figure 5A), while administration of 10 mg/kg/day GSAO caused a >90% inhibition of the rate of tumor growth (Figure 5B). *S.C.* administration of 10 mg/kg/day GSAO inhibited the rate of fibrosarcoma tumor growth in SCID mice (Figure 5C) and Lewis lung (LLC) tumor growth in C57Bl6/J mice (Figure 5D) by ~70%.

Administration of 2 mg/kg/day GSAA had no affect on the rate of BxPC-3 tumor growth while administration of 10 mg/kg/day GSAA resulted in a small inhibition in the rate of tumor growth (<20%) when compared to administration of vehicle alone (not shown). Administration of 10 mg/kg/day GSAA had no affect on the rate of LLC tumor growth in C57Bl6/J mice when compared to administration of vehicle alone (not shown).

There was no apparent adverse affects of administration of either GSAO or GSAA to either SCID or C57Bl6/J mice. The average mice weights of the GSAA and GSAO treatment groups over the course of the experiments were the same (not shown). At the conclusion of the experiment described in Figure 5B all mice were examined. There was no apparent macroscopic differences between the GSAA or GSAO-treated and untreated mice. The heart, lungs, liver, kidneys, and spleen of the GSAA or GSAO-treated and untreated mice were fixed in formaldehyde, embedded in paraffin, sectioned, stained with haematoxylin and eosin and examined by light microscopy. There was no obvious morphological changes in any of the organs of the treated mice when compared to the organs of untreated mice (not shown).

Example 2(h): GSAO Inhibited Angiogenesis in Human Pancreatic Tumors

There was no macroscopic or microscopic signs of necrosis of either GSAA- or GSAO-treated tumors (not shown). Immunohistochemical analysis of the tumors from the experiment described in Figure 5B indicated a significant reduction in blood vessel density in the GSAO-treated tumors ($p<0.001$). An example of the staining for blood vessels is shown in Figure 5E, while the analysis of vessel density in shown in Figure 5F. The proliferative indices of the GSAA- and

GSAO-treated tumors were the same (Figure 5F). There was a significant increase in the apoptotic indices of GSAO- versus GSAA-treated tumors (p=0.05) (Figure 5F).

Example 2(i): GSAO in the Drinking Water Inhibited Human and Murine Primary Tumor Growth

SCID mice bearing *S.C.* AsPC-1 tumors or C57Bl6/J mice bearing *S.C.* LLC tumors were fed either ~10 mg/kg/day GSAO or control GSCA (4-(N-(S-glutathionylacetyl)amino)benzoic acid) or GSAA in their water. The trivalent arsenic in GSAO has been replaced by a carboxylic acid in GSCA. The rate of growth of both tumors was suppressed by ~50% in mice consuming GSAO (Figure 6). The control compounds, GSCA (Figure 6A) or GSAA (Figure 6B), in the water had no significant affect on the rate of growth of either tumor when compared to water alone (not shown).

Example 3 – Discussion

As an exemplerary embodiment, the present invention has targeted PTPases in proliferating endothelial cells with a hydrophilic trivalent arsenical (GSAO). GSAO slowly entered endothelial cells and caused hyperphosphorylation of two focal adhesion proteins, FAK and paxillin.

Focal adhesions are the points of cell adhesion to the extracellular matrix, where integrins and associated signalling proteins are clustered and interact with the cytoskeleton (Giancotti & Ruoslahti, 1999). The structural and signalling components of focal adhesions propagate signals from the integrins to the cytoskeleton and other intracellular components. Integrin adhesion to extracellular matrix leads to an increase in tyrosine phosphorylation of multiple proteins, including FAK and paxillin (Burridge et al., 1992).

FAK colocalizes with integrins in focal adhesions through its C-terminal focal adhesion targeting sequence (Hildebrand et al. 1993). Phosphorylation of FAK leads to recruitment and phosphorylation of downstream targets including the adaptor protein, paxillin (Schaller and Parsons, 1995; Hildebrand et al., 1995). Paxillin becomes phosphorylated on tyrosine in response to integrin-dependent cell adhesion (Bellis et al., 1997). Paxillin interacts with multiple signaling and structural proteins, including PTP-PEST.

Dephosphorylation of FAK and paxillin in endothelial cells is associated with disassembly of focal adhesions and inhibition of dephosphorylation by PAO or vanadate results in increased focal adhesions (Schneider et al., 1998; Retta et al., 1996; Defilippi et al., 1995). FAK is hyperphosphorylated in SHP2 null fibroblasts (Yu et al., 1998) and SHP2 dephosphorylates FAK and paxillin in response to insulin-like growth factor I (Manes et al., 1999). PTEN interacts with and dephosphorylates FAK (Tamura et al., 1998). PTP-PEST and SHP2 are expressed in the blood vessel wall (Wright et al., 2000). PTP-PEST was a PTPase that interacted with relevant

concentrations of GSAO-biotin in endothelial cell lysate. This result implied that inhibition of PTP-PEST by GSAO was primarily responsible for hyperphosphorylation of FAK and paxillin in proliferating endothelial cells. PTP-PEST, in contrast to SHP2 and PTEN, contains an extra cysteine (Cys235) between the catalytic cysteine (Cys231) and the conserved arginine (Arg237) in the active site cleft. An extra cysteine also occurs in a similar position in low molecular weight PTP, which can form a disulfide bond with the catalytic cysteine (Caselli et al., 1998). GSAO probably inhibited PTP-PEST by forming a cyclic dithioarsinite with Cys231 and Cys235.

It is likely that GSAO inhibited other endothelial cell PTPases and increased the phosphorylation state of their substrates. These substrates may have been beyond the resolution of the phosphotyrosine Western blot. The experiment favored the detection of changes in the phosphorylation state of FAK and paxillin as these proteins are two of the most abundant substrates for tyrosine kinases in endothelial cells. For example, PTP-1B, which regulates adhesion, focal adhesion formation and migration in fibroblasts (Liu et al, 1998) and interacts with and dephosphorylates $p130^{Cas}$ (Liu et al, 1996), also bound GSAO-biotin. It is also possible that GSAO perturbed other endothelial cell proteins. PAO binds to and inactivates thioredoxin (Donoghue et al., 2000) and lecithin-cholesterol acyltransferase (Jauhiainen et al., 1988) with dissociation constants of 0.45 μM and ~230 μM, respectively, and inhibits glucose transport (Frost and Lane, 1985) and ubiquitin-dependent protein degradation (Klemperer and Pickart, 1989) with IC_{50} values of 7 μM and 25 μM, respectively. A cytosolic GSAO concentration of ~0.08 μM resulted from 3 days exposure of endothelial cells to 3 μM ^{3}H-GSAO, which was sufficient to inhibit PTPase activity. About 5 to 3,000-fold higher concentrations of PAO than GSAO, therefore, were required to inhibit the activities of these other enzymes and processes. This observation implies that the dominant affect of GSAO in endothelial cells was inhibition of PTPase activity.

GSAO was a selective inhibitor of proliferating endothelial cells but had little affect on growth quiescent endothelial cells or other cell types. The GSAO IC_{50} for inhibition of cell proliferation was 150-400-fold lower for microvascular endothelial cells than for tumor cells, vascular smooth muscle cells or fibroblasts. Similarly, the GSAO IC_{50} for reduction in viability was 20-100-fold lower for microvascular endothelial cells than for the other cells. Inactivation of PTP-PEST by GSAO is predicted to perturb focal adhesion disassembly and was probably a major contributor to the reduction in endothelial cell proliferation and viability. Focal adhesions form in response to growth factors and the need for anchorage-dependent movement and growth. Focal adhesion assembly and disassembly is predicted to be less frequent in growth quiescent endothelial cells, therefore, which may have accounted for the small affect of GSAO on the viability of these cells.

The inhibition of proliferation and reduction in viability of endothelial cells by GSAO *in vitro* correlated with inhibition of angiogenesis *in vivo*. GSAO inhibited angiogenesis in the chick CAM and tumor angiogenesis and tumor growth in mice. S.C. administration of GSAO inhibited the rate of growth of both human or murine tumors in immunocompromised or immunocompetent mice, respectively, by 70 to >90%. There was a marked reduction in blood vessel density in the GSAO-treated BxPC-3 tumors but no signs of necrosis. In addition, there was an increase in the apoptotic index but no change in the proliferative index of the tumor cells. Inhibition of tumor angiogenesis has been associated with an increase in apoptosis of the tumor cells (O'Reilly et al., 1997). The high rate of apoptosis is thought to balance the high proliferative rate of the tumor cells resulting in no net gain in tumor size (Holmgren et al., 1997).

No signs or symptoms of toxicity were observed in any of the *in vivo* experiments. The safety of GSAO in mice was most likely due to its hydrophilicity, which restricts the entry of GSAO into cells, limiting its distribution mostly to the intravascular compartment. This distribution also increases the exposure of proliferating endothelial cells to GSAO. Preliminary studies in mice have shown that GSAO is rapidly excreted in the urine within 6-8 hours of administration and does not appear to accumulate in any organs.

The small size (550 Da) and water solubility of GSAO suggested that it may be orally available in drinking water. The rate of growth of human pancreatic carcinoma and murine LLC tumors in mice was suppressed by ~50% with oral administration of GSAO.

GSAO is the first example of therapeutic intervention of PTPase activity.

Example 4 - Pharmaceutical Formulations

The compounds used in the present invention may be administered alone, although it is preferable that they be administered as a pharmaceutical formulation. The active ingredient may comprise, for topical administration, from 0.001% to 10% by weight, and more typically from 1% to 5% by weight of the formulation, although it may comprise as much as 10% by weight.

In accordance with the best mode of performing the invention provided herein, specific preferred pharmaceutical compositions used in the present invention are outlined below. The following are to be construed as merely illustrative examples of formulations and not as a limitation of the scope of the present invention in any way.

Example 4(a) - Topical Cream Composition

A typical composition for delivery as a topical cream is outlined below:

GSAO	1.0 g
Polawax GP 200	25.0 g

Lanolin Anhydrous	3.0 g
White Beeswax	4.5 g
Methyl hydroxybenzoate	0.1 g
Deionised & sterilised Water to	100.0 g

The polawax, beeswax and lanolin are heated together at 60°C, a solution of methyl hydroxybenzoate is added and homogenisation achieved using high speed stirring. The temperature is then allowed to fall to 50°C. The compound used in the present invention, in this example being GSAO, is then added and dispersed throughout, and the composition is allowed to cool with slow speed stirring.

Example 4(b) - Topical Lotion Composition

A typical composition for delivery as a topical lotion is outlined below:

GSAO	1.2 g
Sorbitan Monolaurate	0.8 g
Polysorbate	20 0.7 g
Cetostearyl Alcohol	1.5 g
Glycerin	7.0 g
Methyl Hydroxybenzoate	0.4 g
Sterilised Water about to	100.00 ml

The methyl hydroxybenzoate and glycerin are dissolved in 70 ml of the water at 75°C. The sorbitan monolaurate, polysorbate 20 and cetostearyl alcohol are melted together at 75°C and added to the aqueous solution. The resulting emulsion is homogenised, allowed to cool with continuous stirring and the GSAO is added as a suspension in the remaining water. The whole suspension is stirred until homogenised.

Example 4(c) - Eye Drop Composition

A typical composition for delivery as an eye drop is outlined below:

GSAO	0.3 g
Methyl Hydroxybenzoate	0.005 g
Propyl Hydroxybenzoate	0.06 g
Purified Water about to	100.00 ml.

The methyl and propyl hydroxybenzoates are dissolved in 70 ml purified water at 75°C, and the resulting solution is allowed to cool. GSAO is then added, and the solution sterilised by filtration through a membrane filter (0.22 μm pore size), and aseptically packed into sterile containers.

Example 4(d) - Composition for Inhalation Administration

For an aerosol container with a capacity of 20-30 ml: a mixture of 10 mg of GSAO with 0.5-0.8% by weight of a lubricating agent, such as polysorbate 85 or oleic acid, is dispersed in a propellant, such as freon, and put into an appropriate aerosol container for either intranasal or oral inhalation administration.

Example 4(e) - Composition for Parenteral Administration

A pharmaceutical composition of the present invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and 1 mg of GSAO.

Similarly, a pharmaceutical composition for intravenous infusion may comprise 250 ml of sterile Ringer's solution, and 5 mg of GSAO.

Example 4(f) - Capsule Composition

A pharmaceutical composition of GSAO in the form of a capsule may be prepared by filling a standard two-piece hard gelatin capsule with 50 mg of GSAO, in powdered form, 100 mg of lactose, 35 mg of talc and 10 mg of magnesium stearate.

Example 4(g) - Injectable Parenteral Composition

A pharmaceutical composition of this invention in a form suitable for administration by injection may be prepared by mixing 1% by weight of GSAO in 10% by volume propylene glycol and water. The solution is sterilised by filtration.

Example 4(h) - Ointment Composition

A typical composition for delivery as an ointment includes 1.0g of GSAO, together with white soft paraffin to 100.0 g, dispersed to produce a smooth, homogeneous product.

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CLAIMS

1. A process for identifying a compound which is a modifier of angiogenesis, said process comprising contacting a cell or cell extract with said compound, determining whether there is a change in the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and thereby determining whether the compound is a modifier of angiogenesis.

2. A process for screening a plurality of compounds to identify a compound which is a modifier of angiogenesis, wherein said process comprises contacting a cell or a cell extract with said plurality of compounds, determining whether there is a change in the activity of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and if so, separately determining the change in activity of said protein tyrosine phosphatase for each of the plurality of compounds, thereby determining the identity of the compound which is a modifier of angiogenesis.

3. The process of claim 1 or 2, wherein said modifier inhibits angiogenesis.

4. The process of claim 1 or 2, wherein said modifier stimulates angiogenesis.

5. The process of claim 1 or 2, wherein said protein tyrosine phosphatase is PTP-PEST.

6. The process of claim 1 or 2, wherein said protein tyrosine phosphatase is PTP-1B.

7. The process according to claim 1 or 2, wherein the cell is a mammalian cell.

8. The process according to claim 7, wherein the cell is selected from the group consisting of: endothelial cells, epithelial cells, immune cells, bone, bone marrow cells and tumour cells.

9. The process according to claim 8, wherein the cell is a proliferating endothelial cell.

10. A method of inhibiting at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B in a vertebrate, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier detected in accordance with the process of claim 3, or a therapeutically effective amount of a pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

11. A method of stimulating at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B in a vertebrate, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier detected in accordance with the process of claim 4, or a therapeutically effective amount of a pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

12. A method of inhibiting angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier detected in accordance with the process of claim 3, or a therapeutically effective amount of a pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

13. A method of stimulating angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier detected in accordance with the process of claim 4, or a therapeutically effective amount of a pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

14. A method of treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one modifier detected in accordance with the process of claim 3, or a therapeutically effective amount of a pharmaceutical composition comprising at least one of said modifiers together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

15. A method of modifying angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and wherein said method comprises administering to the vertebrate a therapeutically effective amount of an compound capable of changing the activity of at least one of said protein tyrosine phosphatases.

16. The method of claim 15, wherein said angiogenesis is inhibited.

17. The method of claim 15, wherein said angiogenesis is stimulated.

18. The method of claim 15 or 16, wherein said activity of said protein tyrosine phosphatase is inhibited.

19. The method of claim 15 or 17, wherein said activity of said protein tyrosine phosphatase is stimulated.

20. A method of treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and wherein said method comprises administering

a therapeutically effective amount of at least one compound capable of inhibiting the activity of at least one of said protein tyrosine phosphatases.

21. A method of treating an angiogenesis dependent disease in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, and wherein said method comprises administering a therapeutically effective amount of at least one compound capable of stimulating the activity of at least one of said protein tyrosine phosphatases.

22. The method of any one of claims 15, 20 or 21, wherein said compound comprises at least one substantially cell-membrane impermeable arsenoxide or arsenoxide equivalent compound.

23. A method of inhibiting angiogenesis in a vertebrate, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase selected from the group consisting of: PTP-PEST and PTP-1B, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one substantially cell-membrane impermeable arsenoxide or arsenoxide equivalent compound.

24. A method of treating an angiogenesis dependent disease, wherein said angiogenesis is dependent on an action of at least one protein tyrosine phosphatase in a vertebrate, wherein said method comprises administering to the vertebrate a therapeutically effective amount of at least one substantially cell-membrane impermeable arsenoxide or arsenoxide equivalent compound.

25. The method according to any one of claims 22, 23 or 24, wherein the substantially cell-membrane impermeable compound is of the formula (I):

$$A\text{-}(L\text{-}Y)_p \qquad (I)$$

wherein

A comprises at least one substantially cell-membrane impermeable pendant group;

L comprises any suitable linker and/or spacer group;

Y comprises at least one arsenoxide or arsenoxide equivalent;

p is an integer from 1 to 10.

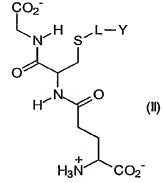
26. The method according to claim 25 wherein the sum total of carbon atoms in A and L together, is greater than 6.

27. The method according to claim 25, wherein A is selected from the group consisting of natural, unnatural and synthetic amino acids, hydrophilic amines, peptides, polypeptides, oligosaccharides, and thiol containing proteins, or a combination thereof.

28. The method according to claim 25, wherein A is selected from the group consisting of glutathione, glucosamine, cysteinylglycine, cysteic acid, aspartic acid, glutamic acid, lysine, and

arginine, and wherein the sulfur atom of each sulfur containing compound may be optionally oxidised to form a sulfoxide or sulfone.

29. The method according to claim 25, wherein A is glutathione and the substantially cell-membrane impermeable compound is represented by Formula (II):



(II)

wherein L comprises any suitable linker and/or spacer group, and Y comprises an arsenoxide or an arsenoxide equivalent.

30. The method according to claim 25, wherein p is an integer from 1 to 5.

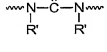
31. The method according to claim 30, wherein p is 1.

32. The method according to claim 25, wherein L corresponds to $(XBX')_nB'$, and wherein n is an integer from 0 to 20,

X is selected from the group consisting of –NR, -S(O)-, -S(O)O-, -S(O)$_2$-, -S(O)$_2$O-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, -P(O)(R_1)-, and -P(O)(R_1)O-, or is absent;

B is selected from the group consisting of C_1-C_{10} alkylene, C_2-C_{10} alkenylene, C_2-C_{10} alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_3-C_{10} heterocycloalkylene, C_5-C_{10} heterocycloalkenylene, C_6-C_{12} arylene, heteroarylene and C_2-C_{10} acyl;

X' is selected from the group consisting of -NR-, –O-, -S-, -Se-, -S-S-, S(O)-, -OS(O)-, OS(O)O-, -OS(O)$_2$, -OS(O)$_2$O-, -S(O)O-, -S(O)$_2$-, -S(O)$_2$O-, -OP(O)(R_1)-, -OP(O)(R_1)O-, -OP(O)(R_1)OP(O)(R_1)O-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, -P(O)(R_1)-, -P(O)(R_1)O-, and



or is absent; wherein E is O, S, Se, NR or N(R)$_2^+$;

and

B' is selected from the group consisting of C_1-C_{10} alkylene, C_2-C_{10} alkenylene, C_2-C_{10} alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_3-C_{10} heterocycloalkylene, C_5-C_{10} heterocycloalkenylene, C_6-C_{12} arylene, and heteroarylene or is absent; and wherein

each R is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, OR_2 and C_2-C_{10} acyl;

R' is the same as R or two R' may be taken together with the nitrogen atoms to which they are attached to form a 5 or 6-membered saturated or unsaturated heterocyclic ring;

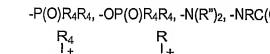
each R_1 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, halo, OR_2 and $N(R)_2$;

each R_2 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl and $-C(O)R_5$;

each R_5 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, OH, SH and $N(R)_2$;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent) may be in a para-, meta- or ortho- relationship; and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, heterocycloalkylene, heterocycloalkenylene, arylene, heteroarylene and acyl may be independently substituted with hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, cyano, cyanate, isocyanate, OR_{2a}, SR_6, nitro, arsenoxide, $-S(O)R_3$, $-OS(O)R_3$, $-S(O)_2R_3$, $-OS(O)_2R_3$, $-P(O)R_4R_4$, $-OP(O)R_4R_4$, $-N(R'')_2$, $-NRC(O)(CH_2)_mQ$, $-C(O)R_5$;



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)(R_4)_2$, $N(R)_2$ and $-C(O)R_5$;

each R_3 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio and $N(R)_2$;

each R_4 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, halo and $N(R)_2$;

R_6 is selected from the group consisting of C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, $-S(O)R_3$, $-S(O)_2R_3$ and $-C(O)R_5$;

R" is the same as R or two R" taken together with the N atom to which they are attached may form a saturated, unsaturated or aromatic heterocyclic ring system;

Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

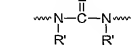
m is 1 to 5.

33. The method according to claim 25, wherein

X is selected from the group consisting of -C(O)-, -C(S)-, -C(O)O-, C(S)O-, and -C(S)S-, or is absent;

B is selected from the group consisting of C_1-C_5 alkylene, C_2-C_5 alkenylene, C_2-C_5 alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_6-C_{12} arylene and C_2-C_5 acyl;

X' is selected from the group consisting of –O-, -S-, -NR-, -S-S-, -S(O)-, -S(O)$_2$-, -P(O)(R_1)-, -OP(O)(R_1)-, OP(O)(R_1)O-, -OP(O)(R_1)OP(O)(R_1)O-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, -Se-,



, or is absent; wherein E is O, S or $N(R)_2$;

n is 0, 1 or 2; and

B' is C_1-C_5 selected from the group consisting of alkylene, C_2-C_5 alkenylene, C_2-C_5 alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, and C_6-C_{12} arylene, or is absent; and wherein

each R is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, OR_2 and C_2-C_{10} acyl;

R' is the same as R;

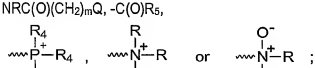
each R_1 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, OR_2 and $N(R)_2$;

each R_2 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, and -C(O)R_5;

each R_5 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, OH, SH, and $N(R)_2$;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent), may be in a para-, meta- or ortho- relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, cyano, halo, cyanate, isocyanate, OR_{2a}, SR_5, nitro, arsenoxide, -S(O)R_3, -OS(O)R_3, -$S(O)_2R_3$, -OS$(O)_2R_3$, -P(O)R_4R_4, -OP(O)R_4R_4, -$N(R'')_2$, NRC(O)$(CH_2)_mQ$, -C(O)R_5,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, -S(O)R_3, -$S(O)_2R_3$, -P(O)$(R_4)_2$, $N(R)_2$ and -C(O)R_5;

each R_3 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5

alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio and $N(R)_2$;

each R_4 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_5 cycloalkylthio, C_5-C_5 cycloalkenylthio, C_6-C_{12} arylthio, halo and $N(R)_2$;

R_5 is independently selected from the group consisting of C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, $-S(O)R_3$, $-S(O)_2R_3$ and $-C(O)R_5$,

R" is the same as R;

Q is selected from the group consisting of halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

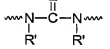
m is 1 to 5.

34. The method according to claim 25, wherein

X is absent;

B is selected from the group consisting of C_1-C_5 alkylene, C_6-C_{12} arylene and C_2-C_5 acyl;

X' is selected from the group consisting of –O-, -S-, -NR-, -S-S-, -S(O)-, $-S(O)_2$-, $-P(O)(R_1)$-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -Se-, and



, or absent; wherein E is O, S or $N(R)_2^+$;

n is 0, 1 or 2; and

B' is C_1-C_5 alkylene, C_6-C_{12} arylene or is absent; and wherein

each R is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, OR_2 and C_2-C_5 acyl;

R' is the same as R;

each R_1 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, halo, OR_2 and $N(R)_2$;

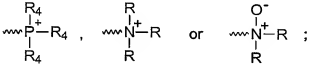
each R_2 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl and $-C(O)R_5$;

each R_5 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_{10}

cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, OH, SH and $N(R)_2$;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent) may be in a para-, meta- or ortho- relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, cyano, cyanate, isocyanate, OR_{2a}, SR_6, nitro, arsenoxide, $-S(O)R_3$, $-OS(O)R_3$, $-S(O)_2R_3$, $-OS(O)_2R_3$, $-P(O)R_4R_4$, $-OP(O)R_4R_4$, $-N(R'')_2$, $-NRC(O)(CH_2)_mQ$, $-C(O)R_5$,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)(R_4)_2$ and $-C(O)R_5$;

each R_3 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_{10} cycloalkyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_{10} cycloalkylthio, C_6-C_{12} arylthio and $N(R)_2$;

each R_4 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_{10} cycloalkyloxy, C_6-C_{12} aryloxy, halo and $N(R)_2$;

R_6 is selected from the group consisting of C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_3-C_{10} cycloalkylthio, C_6-C_{12} arylthio, $-S(O)R_3$, $-S(O)_2R_3$ and $-C(O)R_5$,

R'' is the same as R;

Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

35. The method according to claim 25, wherein

X is absent;

B is selected from the group consisting of C_1-C_5 alkylene, C_6-C_{12} arylene and C_2-C_5 acyl;

X' is selected from the group consisting of –O-, -S-, -NR-, -C(O)-, and -C(O)O-, or is absent;

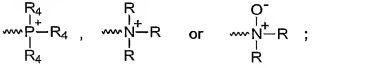
n is 1; and

B' is C_1-C_5 alkylene, C_6-C_{12} arylene or is absent; and

R is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl and C_2-C_5 acyl;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent), may be in a para-, meta- or ortho- relationship, and

wherein each alkylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, cyano, cyanate, isocyanate, OR_{2a}, SR_6, nitro, arsenoxide, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)R_4R_4$, $-N(R'')_2$, $-NRC(O)(CH_2)_mQ$, $-C(O)R_5$,



wherein each R is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl and C_2-C_5 acyl;

R_{2a} is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)(R_4)_2$ and $-C(O)R_5$;

each R_3 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, and C_6-C_{12} arylthio;

each R_4 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_6-C_{12} arylthio, halo and $N(R)_2$;

each R_5 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_6-C_{12} arylthio, OH, SH and $N(R)_2$;

R_6 is selected from the group consisting of C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_6-C_{12} arylthio, $-S(O)R_3$, $-S(O)_2R_3$ and $-C(O)R_5$,

R'' is the same as R above;

Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

36. The method according to claim 25, wherein

X is absent;

B is C_2-C_5 acyl;

X' is NR;

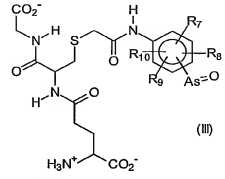
n is 1;

B' is phenylene; and

R is H;

wherein the substituents directly attached to the phenylene ring may be in a para-, meta- or ortho- relationship.

37. The method according to claim 25, wherein the compound is represented by Formula (III):



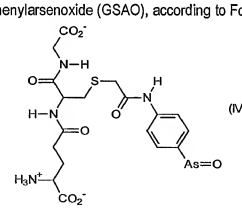
(III)

wherein R_7 to R_{10} are independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, $-OS(O)_2R_3$ and $-NHC(O)CH_2Q$ wherein Q is halogen, $-OS(O)_2CH_3$, $-OS(O)_2C_6H_5$ and $-OS(O)_2$-*p* tolyl.

38. The method according to claim 37, wherein R_7 to R_{10} are independently selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, cyano, carboxy, C_1-C_5 alkoxy, methyl, ethyl, isopropyl, tert-butyl, phenyl and $-NHC(O)CH_2Q$ wherein Q is halogen, $-OS(O)_2CH_3$, $-OS(O)_2C_6H_5$ and $-OS(O)_2$-*p* tolyl.

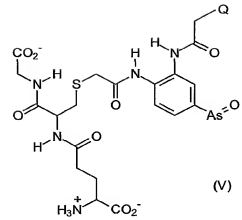
39. The method according to claim 37, wherein the arsenoxide (-As=O) group is in the 4-position of the phenylene ring.

40. The method according to claim 25, wherein said compound is 4-(*N*-(*S*-glutathionylacetyl)amino)-phenylarsenoxide (GSAO), according to Formula IV:



(IV)

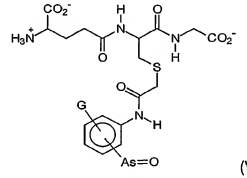
41. The method according to claim 25, wherein said compound is represent by Formula (V):



(V)

wherein Q is any halogen.

42. The method according to claim 25, wherein the compound is represented by Formula (VI):



(VI)

wherein G is selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, cyano, carboxy, C_1-C_5 alkoxy, C_1-C_5 alkyl and C_6-C_{12} aryl and -NHC(O)CH_2Q, wherein Q is halogen, -OS(O)$_2CH_3$, -OS(O)$_2C_6H_5$ or –OS(O)$_2$-*p* tolyl.

43. The method according to claim 42, wherein G is selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, methyl, ethyl, iso-propyl, tert-butyl, phenyl, and -NHC(O)CH_2Q, wherein Q is the group consisting of halogen, -OS(O)$_2CH_3$, -OS(O)$_2C_6H_5$ and –OS(O)$_2$-*p* tolyl.

44. The method according to claim 43, wherein G is selected from hydroxy, fluorine, amino and nitro.

45. The method according to claim 44, wherein the activity of the arsenic atom is modified by the group G, when G is in an ortho- or para- relationship to the arsenoxide group.

46. The method according to claim 25, the arsenoxide group (-As=O) is replaced by an arsenoxide equivalent.

47. The method according to claim 46, wherein said arsenoxide equivalent is of the form -D(Z_1)(Z_2), wherein D is selected from As, RSn, Sb, or RGe, and Z_1 and Z_2, and wherein Z_1 and Z_2

are identical or different, and are connected or independent from each other (bound only to the arsenic atom).

48. The method according to claim 47, wherein Z_1 and Z_2 are selected from the group consisting of OH, C_1-C_{10} alkoxy, C_6-C_{10} aryloxy, C_1-C_{10} alkylthio, C_6-C_{10} arylthio, C_1-C_{10} alkylseleno, C_6-C_{10} arylseleno, F, Cl, Br and I; and wherein R is an alkyl or an aryl group.

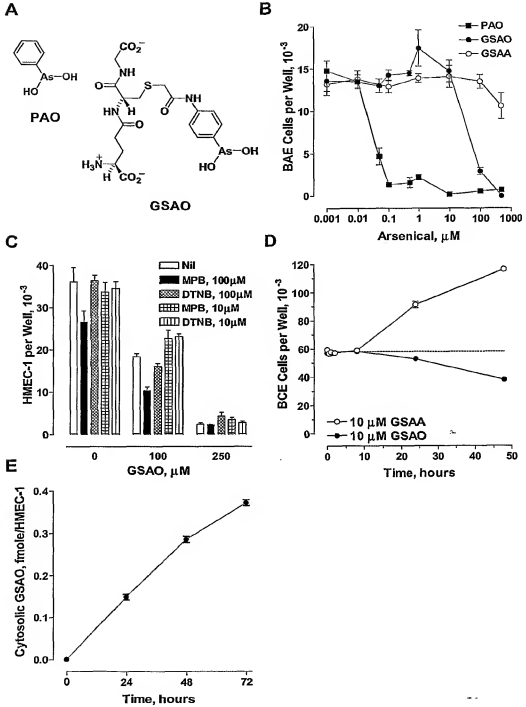

A
PAO
GSAO
B
PAO
GSAO
GSAA
BAE Cells per Well, 10-3
Arsenical, μM
C
Nil
MPB, 100μM
DTNB, 100μM
MPB, 10μM
DTNB, 10μM
HMEC-1 per Well, 10-3
GSAO, μM
D
BCE Cells per Well, 10-3
10 μM GSAA
10 μM GSAO
Time, hours
E
Cytosolic GSAO, fmole/HMEC-1
Time, hours

Figure 1

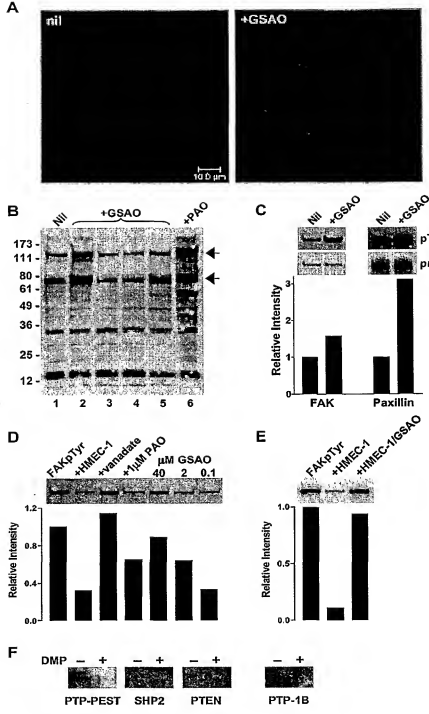

A
nil
+GSAO
100 µm
B
Nil
+GSAO
+PAO
173
111
80
61
49
36
25
12
1 2 3 4 5 6
C
Nil
+GSAO
Nil
+GSAO
pTyr
protein
Relative Intensity
FAK
Paxillin
D
FAKpTyr
+HMEC-1
+vanadate
+1µM PAO
µM GSAO
40 2 0.1
Relative Intensity
E
FAKpTyr
+HMEC-1
+HMEC-1/GSAO
Relative Intensity
F
DMP
PTP-PEST
SHP2
PTEN
PTP-1B

Figure 2

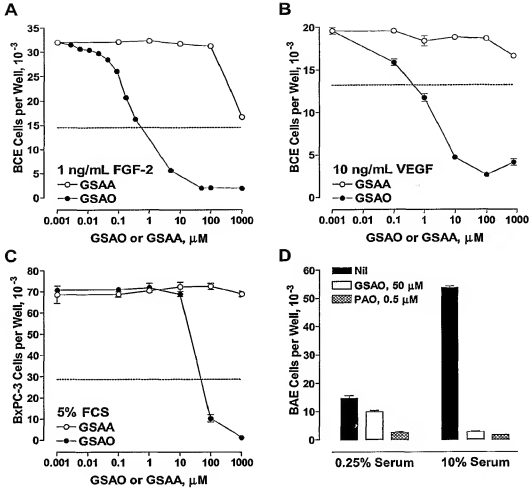

A
BCE Cells per Well, 10-3
1 ng/mL FGF-2
GSAA
GSAO
GSAO or GSAA, µM
B
BCE Cells per Well, 10-3
10 ng/mL VEGF
GSAA
GSAO
GSAO or GSAA, µM
C
BxPC-3 Cells per Well, 10-3
5% FCS
GSAA
GSAO
GSAO or GSAA, µM
D
BAE Cells per Well, 10-3
Nil
GSAO, 50 µM
PAO, 0.5 µM
0.25% Serum
10% Serum

Figure 3

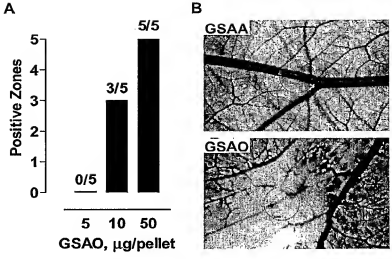

A
B
Positive Zones
5
4
3
2
1
0
0/5
3/5
5/5
5
10
50
GSAO, µg/pellet
GSAA
GSAO

Figure 4

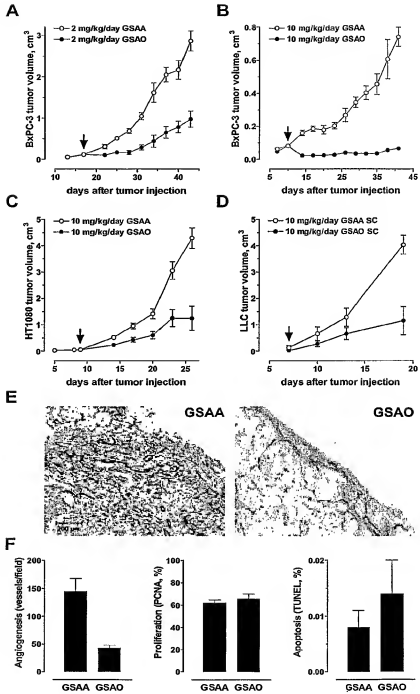

A
2 mg/kg/day GSAA
2 mg/kg/day GSAO
BxPC-3 tumor volume, cm3
days after tumor injection
B
10 mg/kg/day GSAA
10 mg/kg/day GSAO
BxPC-3 tumor volume, cm3
days after tumor injection
C
10 mg/kg/day GSAA
10 mg/kg/day GSAO
HT1080 tumor volume, cm3
days after tumor injection
D
10 mg/kg/day GSAA SC
10 mg/kg/day GSAO SC
LLC tumor volume, cm3
days after tumor injection
E
GSAA
GSAO
F
Angiogenesis (vessels/field)
Proliferation (PCNA, %)
Apoptosis (TUNEL, %)
GSAA GSAO
GSAA GSAO
GSAA GSAO

Figure 5

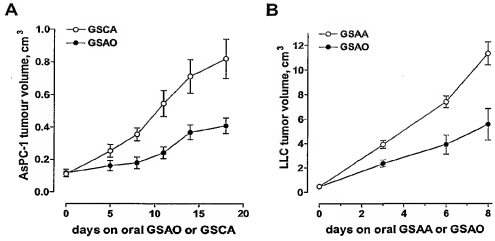

A
GSCA
GSAO
AsPC-1 tumour volume, cm³
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
days on oral GSAO or GSCA
B
GSAA
GSAO
LLC tumor volume, cm³
12
10
8
6
4
2
0
0
2
4
6
8
days on oral GSAA or GSAO

Figure 6

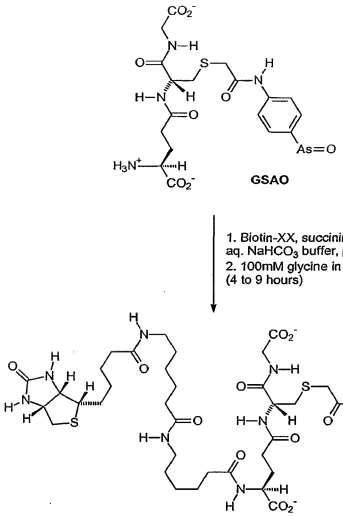

GSAO
1. Biotin-XX, succinimidyl ester in DMSO, aq. NaHCO3 buffer, pH 9.6 (60 to 90 mins).
2. 100mM glycine in PBS buffer, pH 7.3 (4 to 9 hours)
GSAO-B

FIGURE 7

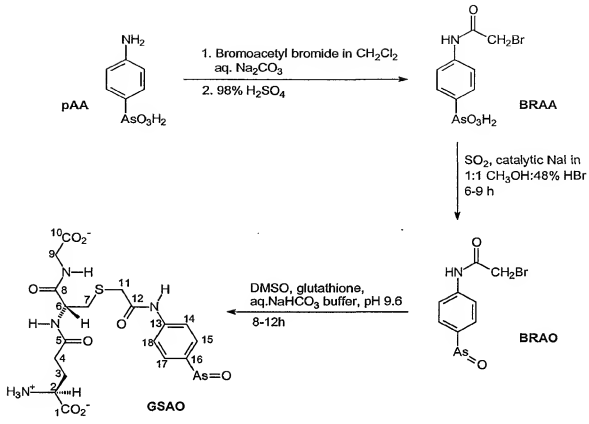

pAA
1. Bromoacetyl bromide in CH_2Cl_2
aq. Na_2CO_3
2. 98% H_2SO_4
BRAA
SO_2, catalytic NaI in
1:1 CH_3OH:48% HBr
6-9 h
BRAO
DMSO, glutathione,
aq.$NaHCO_3$ buffer, pH 9.6
8-12h
GSAO

FIGURE 8

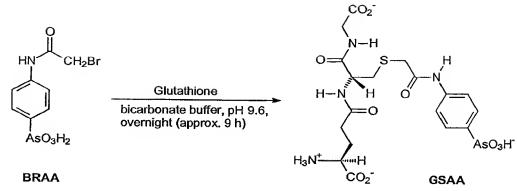

Glutathione
bicarbonate buffer, pH 9.6,
overnight (approx. 9 h)
BRAA
GSAA

FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/00848

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: G01N 33/53; C12Q 1/42; A61P 9/00, 35/00, 43/00; A61K 33/36, 38/06.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC G01N 33/53; C12Q 1/42; A61P 9/00, 9/14, 35/00, 43/00; A61K 33/36, 38/06

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Derwent (WPAT and JAPIO) Chemical Abstracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/21628 A1 (Unisearch Limited) 29 March 2001 (see entire document, in particular page 15 lines 15-18, page 33 line 16 to page 34 line 30, page 60 lines 15-30, page 62 line 14 to page 63 line 5, Figures 22 and 26, Claims 1-29 and 40-42)	1, 3, 5-10, 12, 14-16, 18, 20, 22-48
X	WO 99/61467 A3 (McGill University) 2 December 1999 (see entire document, in particular page 3 line 37, page 41 line 21, page 42 lines 1-3 and claim 10)	15-16, 18, 20
X	US 5753687 A (Mjalli, A. *et al.*) 19 May 1998 (see entire patent, in particular column 9 lines 49-57 and columns 78-82)	1, 6, 10-11

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 September 2002	7 OCT 2002
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	NORMAN BLOM Telephone No : (02) 6283 2238

Form PCT/ISA/210 (second sheet) (July 1998)

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5965558 A (Mjalli, A. *et al.*) 12 October 1999 (see entire patent, in particular column 9 line 52 to column 10 line 26, column 97-column100)	1, 3-4, 6, 10-11
X	Bioorganic & Medicinal Chemistry (1998), **6**, 1457-1468, "Potent Non-peptidyl Inhibitors of Protein Tyrosine Phosphatase 1B". S. D. Taylor *et al.* (see entire document, in particular page 1458)	1, 3, 6
X	The Journal of Biological Chemistry (1988), **263** (14), 6731-6737, "Characterization of the Major Protein-tyrosine-phosphatases of Human Placenta" (see the entire document, in particular page 6733 column 2 to page 6735 column 2)	1, 3-4, 6-7
X	US 5798374 A (Tang, P.C. and McMahon, G.) 25 August 1998 (see in particular columns 3 to 5 and columns 8 to 10)	1, 3-4, 6-7, 10-11
A	WO 01/12180 A2 (UNIVERSITÉ LAVAL) 22 February 2001 (see entire document, in particular page 1 line 15 to page 4 line 27, page 14 lines 3-9)	1-24
A	WO 00/15258 A1 (Vanderbilt University) 23 March 2000 (see entire document, claims)	1-24
A	Biochimica et Biophysica Acta (1994), **1221**, 73-77, "Thioallyl compounds: Potent inhibitors of cell proliferation", E. S. Lee *et al* (see entire document, in particular abstract and pages 76-77)	1-9
A	Biopolymers (Peptide Science) (1998), **47**, 225-241, "Protein-Tyrosine Phosphatases: Structure, Mechanism, and Inhibitor Discovery", T. R. Burke Jr., and Zhong-Yin Zhang (see entire document, in particular page 231 column 2, page 233 column 1 and page 237)	1-9
A	EP 1046715 A1 (Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.) 25 October 2000 (see entire document, in particular Claims 6-7 and 11, paragraphs [0005], [0006], [0023])	1-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU02/00848

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	01/21628	AU	2967/99	EP	1228076		
WO	99/61467	AU	39229/99	CA	2329157	EP	1077997
EP	1046715	AU	45543/00	WO	00/65085	EP	1173608
WO	00/15258	AU	57977/99	EP	1109578	US	6248327
US	5753687	US	5770620	US	595558	US	6150532
		US	6388076	AU	77358/96	AU	15667/97
		CA	2224874	EP	833629	EP	946518
		WO	97/08934	WO	98/27065		
US	596558	US	5770620	US	5753687	US	6150532
		AU	77358/96	AU	15667/97	CA	2224874
		EP	833629	EP	946518	WO	97/08934
		WO	98/27065				
US	5798374	AU	57103/98	AU	62671/96	CA	2192796
		CN	1184635	HU	9603484	NO	965376
		WO	96/40129	WO	98/27092	US	5883110
		US	6080772	US	6143765		
WO	01/12180	AU	65509/00	BR	200013147	EP	1202728

END OF ANNEX